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PECULIARITIES OF THE PIGMENT COMPOSITION AND MORPHOLOGY OF THE PHOTOSYNTHETIC APPARATUS IN CHLOROPHYLL-DEFICIENT LINSEED PLANTS

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ABSTRACT

The pigment composition and morphology of photosynthetic apparatus in chlorophyll-deficient plants of *viridis* and *xantha* types in comparison with green plant is studied. The plants at the stage of 8-10 true leaves of linseed variety Tsian and M-80 (*viridis*) and M-81 (*xantha)* chlorophyll-deficient mutant lines obtained through induced mutagenesis on its basis were used as a material. The plants were grown in the field conditions of the Steppe zone of Ukraine. The leaves of *viridis* chlorophyll-deficient mutant had a decrease in the amount of chlorophyll *a* and catastrophic shortage – chlorophyll *b*. However these mutant plants produced the amount of carotenoids at the level of control green plant. The morphology of their chloroplasts had no any differences as well. *Xantha* chlorophyll-deficient mutant was characterized by a significant decrease in amount of all the test pigments compared to the control. It also had a change in the shape of chloroplasts which affected their linear dimensions and derivates-volume. A different mechanisms of adaptation in the mutants with different type of chlorophyll deficiency are considered.

Keywords: linseed, leaf chlorophyll-deficiency, photosynthetic pigments, chloroplast, morphology.

INTRODUCTION

Plant growth due to photosynthesis. The identification of anatomical and morphological features of the photosynthetic apparatus is of great importance in the study of the mechanisms of photosynthesis and the adaptability of plants to the habitat. At the biochemical level, the chlorophyll molecule basic unit of photosynthesis is, and on the anatomical one - the chloroplasts

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(1, 2). A convenient model for studying the processes of photosynthesis and structures that take part in it directly are chlorophyll mutants (3, 4).

The genetic collection of linseed in Zaporozhye National University contains samples of chlorophyll mutants with different appearance of chlorophyll deficiency – *albina*, *viridis*, *xantha*, *xantho-viridis*. These mutants were isolated by treating linseed seeds (*Linum humile* Mill.) with mutagens of various nature. In this case, the type of chlorophyll deficiency is determined even in the early stages of plant ontogeny (3, 4).

Linseed is a valuable oilseed and technical crop, which is a good precursor for many crops. The plant has a high level of productivity. The life cycle of linseed includes the following stages of development: seedlings (cotyledon leaves appear on the surface of the soil), stage of 8-10 leaves (from the appearance of true leaves to budding), budding, flowering and ripening (5).

Adaptations of leaf, cells and chloroplasts can reflect the mechanism of plant adaptation to water, light and salt regimes. Morphological studies of these adaptations make it possible to understand the ways of adaptation of plants to habitat conditions, to assess plasticity and potential functional capabilities (6).

With a comprehensive study of the photosynthetic apparatus, the pigment content can serve as a physiological and biochemical index for characterizing the functional activity. The pigment content is one of the most important indicators of the photosynthetic apparatus of plants; by their ratio, one can speak of the degree of development of the photosynthetic apparatus and the physiological state of the plant, of the potential to assimilate CO_2 and form a yield under different growing conditions and at different stages of development (7).

The purpose of this study was to study the content of the main photosynthetic pigments and the morphological structure of the photosynthetic apparatus of chlorophyll-deficient linseed plants with different types of chlorophyll deficiency at the stage of 8-10 leaves, in comparison with the initial green plant.

MATERIALS AND METHODS

The plants at the stage of 8-10 true leaves of linseed variety Tsian and M-80 (*viridis*) and M-81 (*xantha*) chlorophyll-deficient mutant lines obtained through induced mutagenesis on its basis were used as a material (3, 4). The plants were grown in the field conditions of the Steppe zone of Ukraine.

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a) b) c)

Fig 1: Linseed variety Tsian (a) and its chlorophyll-deficient mutants M-80 (b) and M-81 (c) at the stage of 8-10 leaves

In the control variety, the leaves had a green color along the entire height of the stem. The mutant line M-80 was characterized by a light green color of the leaves in the upper part of the plant and was referred to the chlorophyll deficiency of *viridis* type. The mutant line M-81 has a chlorophyll deficiency of the *xantha* type. It is characterized by a bright yellow leaves in the upper part of the plant, while the basal part has a light green color.

To determine the content of photosynthetic pigments (chlorophyll *a*, *b* and carotenoids), the leaves were selected both in the chlorophyll-deficient and green parts of the mutants. The leaves of Tsian variety, selected in the same parts, were used as control.

The leaf weighed thoroughly in a mortar with a small amount of CaCO3, quartz sand and 80% acetone. The homogenate was filtered on a folded filter to extract the photosynthetic pigments.

To calculate the concentration of chlorophyll *a*, chlorophyll *b* and carotenoids, the optical density of the extract was determined on a spectrophotometer at a wavelength corresponding to the maximum absorption of the investigated pigments in a given solvent, λ = 663, 646, and 470 nm. Control - pure solvent (80% acetone), l cuv. = 1 cm. The concentration of pigments was calculated by the formula for the determination of chlorophylls and carotenoids in 80% acetone (8, 9).

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To study the morphological features of cells and chloroplasts in a suspension culture, the A. T. Mokronosov method was used (10). The cuttings derived from the upper leaves of the Tsian variety and the chlorophyll-deficient parts of the mutants, were made, placed in a 20% solution of NaOH (with an approximate calculation of 2 cm^2 of leaves in 1 ml of alkali). In the flame of the burner, the solution with the discs was brought to a boil, after which the cells were macerated by grinding the mixture with a glass rod until a homogeneous suspension was obtained.

The resulting preparations were photographed with the XS-3330 trinocular microscope and the MA88-500 eyepiece chamber with an increase of ×640. The dimensions of chloroplasts and cells (length and width) were measured by standard methods using an eyepiece micrometer (11). The shape of chloroplasts was determined according the ratio of length and width. The value of more than 2.5 corresponds to the shape of the cylinder, and less – of the ellipse. The cross-sectional area and the volume of chloroplasts and cells were calculated using the A. T. Mokronosov technique (10, 12)

The results were processed using methods of standard mathematical statistics.

RESULTS

In linseed variety Tsian the amount of chlorophyll *a* was 754.7 ± 63.77. The amount of chlorophyll *b* and carotenoids was at one level and was approximately one third of the chlorophyll *a* content (Fig 2).

Characterizing M-80 mutant sample based on the content of photosynthetic pigments in a leaves with chlorophyll deficiency, it should be noted that the amount of chlorophyll *b* is significantly reduces. The amount of carotenoids is half that of chlorophyll *a*. The content of photosynthetic pigments in the green leaves had the same ratio as in the control sample.

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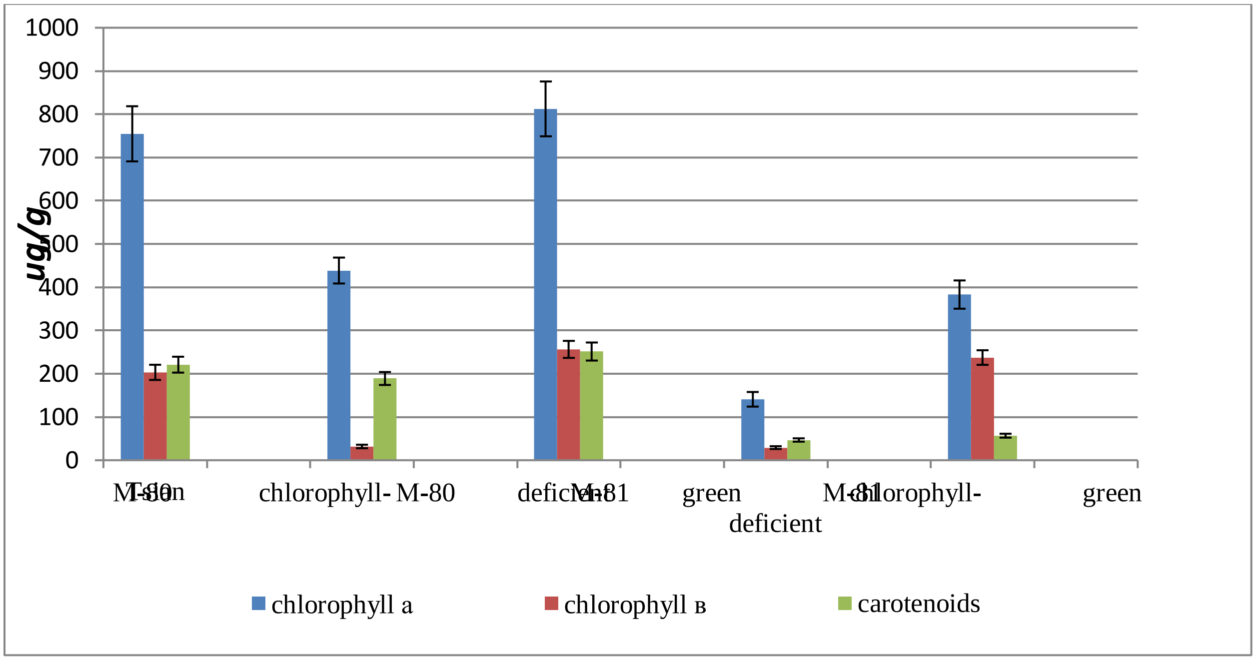
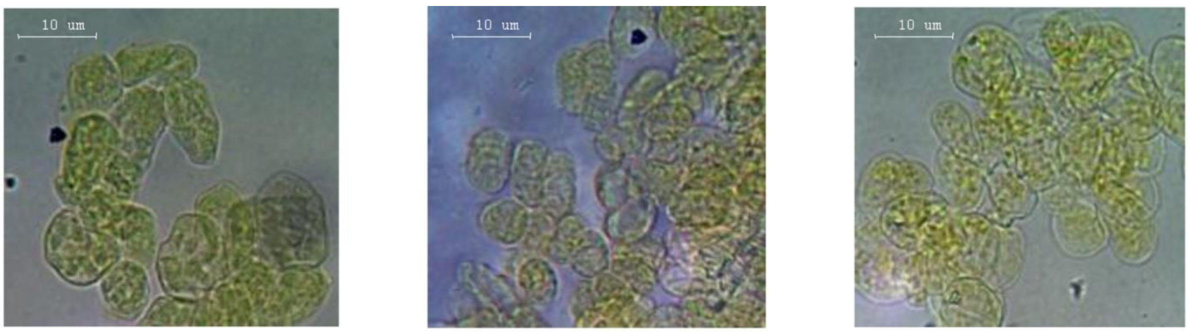


Fig 2: The content of main photosynthetic pigments in chloroplasts of linseed variety Tsian and its mutants in chlorophyll-deficient and green parts of plant

In M-80 mutant is a leaves with chlorophyll deficiency of *viridis* type, the chlorophyll *a* content was almost twice as low as that of the Tsian variety. The amount of chlorophyll *b* is four times smaller, and the content of carotenoids was at the same level. The amount of photosynthetic pigments in the green leaves of the lower part of plant was at the level of the control Tsian variety.

Describing the M-81 mutant sample for the content of photosynthetic pigments, it should be noted that chlorophyll *a* is prevalent in the chlorophyll-deficient leaves of the upper part and green leaves of the lower part of plant, while the quantity of carotenoids remains the same. As for chlorophyll *b*, its ratio substantially changes in the leaves of various parts of the plant. So in a leaves with chlorophyll deficiency the amount of chlorophyll *a* exceeds the amount of chlorophyll *b* three times. In the light green leaves of the lower part of plant, the amount of chlorophyll *b* increases. But the quantity of both chlorophylls grows more than twice, when comparing leaves with chlorophyll deficiency and green leaves of the lower part of plant.

The amount of photosynthetic pigments in the M-81 mutant compared to the Tsian variety is much lower. So in the leaves with chlorophyll deficiency the content of the investigated pigments in comparison with the control is four times smaller. In the green leaves of the lower part of the mutant plant, the ratio changes due to chlorophyll *b*, which increases to the control level. The amount of chlorophyll *a* is less than the Tsian sample twice, and the carotenoids content is fourfold.



a) b) c)

Fig 3: Morphology of chloroplasts in suspension cells of linseed variety Tsian (a) and chlorophyll-deficient part of mutants M-80 (b) and M-81 (c) at the stage of 8-10 leaves

Analyzing the chloroplasts of the control sample (Tsian variety) in the cells of the suspension culture, it was revealed that they had the shape of an ellipse and completely filled the cell. The same pattern was observed in the M-80 chlorophyll mutant studied. In M-81 chlorophyll-deficient mutant with chlorophyll-deficiency of *xantha* type the shape of chloroplasts changes, acquiring the shape of a cylinder.

There were no significant differences in the length and width of cells, in the suspension culture, between the genotypes under investigation. Analyzing the size of the cross-sectional area of the cells, it can be observed that the mutant M-80 tends to decrease of this index. As for the volume of cells, the M-80 mutant shows a decrease in this index. In a chlorophyll-deficient M-81 sample, the cell volume does not differ significantly from the size of the control.

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Table 1: Morphological characteristics of cells and their chloroplasts in linseed variety Tsian and its mutants in chlorophyll-deficient parts of the plant

Genotype	Cells			Chloroplasts			
	length, um	width, um	cross-sectional area, um^2	length, um	width, um	cross-sectional area, um^2	shape of chloroplasts
Tsian	9,06 ±1,192	6,53 ±0,948	166,56 ±10,739	2,47± 0,151	2,01 ±0,098	14,55 ±1,643	ellipse
M-80	8,37 ±1,264	5,78 ±1,372	134,27 ±11,597	2,14 ±0,135	1,89 ±0,107	12,18 ±1,951	ellipse
M-81	8,64 ±1,081	6,63 ±1,083	164,67± 16,973	2,74 ±0,169	1,18 ±0,196*	9,32 ±2,174	cylinder

* - differences from the Tsian variety are significant at $p < 0,01$

Studying the length of the chloroplasts in suspension cells at the top of the control sample and the M-80 and M-81 mutant lines, significant differences were not found from the control. With regard to the width of the chloroplasts, Table 1 shows that the mutant of *viridis* type did not differ significantly from the control. In M-81 mutant with chlorophyll deficiency of *xantha* type with the lightest color of the leaves, the width of the chloroplasts was almost halved. Therefore, this mutant exhibits a change in the ratio of length and width of chloroplasts (l/d => 2.5), as a result of which the shape of chloroplasts was defined as a cylinder. In the control sample with green leaves, an ellipsoidal shape of chloroplasts was observed for all the investigated parts of the plant. Based on the obtained parameters of the length and width of the chloroplasts, and also taking into account their shape, such chloroplast indexes as the cross-sectional area and volume were calculated. For ellipsoidal chloroplasts, the formula of the ellipse was used, and for the characterization of the chloroplasts in the M-81 mutant line, the cylinder formula was used. The cross-sectional area in the chlorophyll-deficient mutants did not differ significantly from the control parameters. When calculating the volume of chloroplasts, it was found that the volume of chloroplasts significantly decreases in the M-81 mutant.

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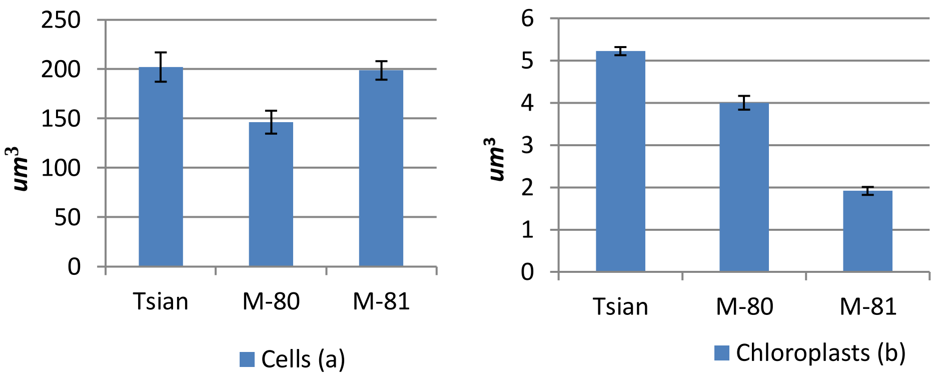

Fig 4: The volume of cells (a) and their chloroplasts (b) in linseed variety Tsian and its mutants in chlorophyll-deficient parts of plant

Summarizing the data obtained it should be noted that in the suspension culture cells, differences in the morphological structure of the photosynthetic apparatus of chlorophyll-deficient and green leaves are more to be found not between linear dimensions, but derived indicators like shape and volume. It was revealed that in the mutant samples the volume of chloroplasts and cells decreases in comparison with the control. The mutant M-81 with chlorophyll defficiency of the *xantha* type exhibits a change in the shape of the chloroplasts.

DISCUSSION

The main role in the conversion of physical energy into chemical belongs to a complex of photosynthetic pigments, primarily chlorophyll. Pigment plastids are the main components of the photosynthetic apparatus of plants, which are responsible for absorbing the energy of light and its transformation into the energy of chemical bonds (13). The physiological state of plants under different environmental conditions depends on the content of photosynthetic pigments. The absolute composition of pigments and their ratio in any plant is not constant. It can vary considerably depending on the intensity and quality of light, climatic conditions, the structure of the leaf blade, etc (7).

We studied the content of chlorophylls and carotenoids in Tsian variety in the leaves of the upper and lower parts of the plant at the 8-10 leaf stage. There was no significant difference between the indices of the number of basic photosynthetic pigments. Perhaps this is due to the fact that in this case all the leaves were of the same age. From the literature data it is known that in flax plants the amount of photosynthetic pigments can increase to the flowering stage (5). At the same time, our previous studies indicate that in linseed variety Tsian in juvenile leaves at the stage of budding and definitive leaves at the flowering stage, no significant differences in the number of photosynthetic pigments were observed (14).

In our study it was also found that the control sample (Tsian variety) had a high chlorophyll *a* content, while the amount of chlorophyll *b* was three times less. It is known that the content of chlorophylls *a* and *b* in leaves has the opposite tendency. For example, as in our case, in the leaves of wheat the high content of chlorophyll *a* corresponded to a very small amount of chlorophyll *b*. But it is possible and vice versa (15). All the linseed samples we studied showed a low chlorophyll content *b*. It can be assumed that linseed plants are deficient in chlorophyll *b*, since this feature does not depend on environmental factors and is appeared in all genotypes.

Pigments are very sensitive to the environment, which causes high phenotypic plasticity of plants (16). In the absence of chlorophyll *b*, structurally functional rearrangements of the photosynthetic apparatus can occur, which does not interfere with achieving high productivity in the subsequent stages of ontogeny (17). For example, important for the integrity of the photosynthetic apparatus are carotenoids, which can perform a protective function against the destruction of chlorophylls (13,18, *19*).

In our case, in the control sample, the level of carotenoids reached the level of chlorophyll *b*. The same ratio of pigments can be observed in the green leaves of the mutant M-80 with chlorophyll deficiency of the *viridis* type. The leaves of the chlorophyll-deficient part of this mutant with a decrease in the amount of chlorophyll *a* and the catastrophic shortage of chlorophyll *b* had the same amount of carotenoids as Tsian variety. This exceeds the content of chlorophyll *b* by more than 4 times. An increase in the number of carotenoids against the background of a decrease in chlorophyll *b* is probably an adaptive mechanism for stabilizing the process of photosynthesis in mutants of *viridis* type in their chlorophyll-deficient part of the plant. Since the lower leaves of this mutant have no significant differences from the control, it should be assumed that the process of photosynthesis in them does not have significant changes and contributes to the normalization of the development of mutant plants with chlorophyll deficiency of *viridis* type as a whole.

As is known in pea plants, the amount of pigments differs and can be the opposite, depending on the period of plant growth. So in May the quantity of pigments can have a higher level than in June-July, as the amount of light increases. Therefore, a low chlorophyll content is a positive factor for the effective functioning the photosynthetic apparatus (20). A decrease in the amount of chlorophyll in chlorophyll *b* mutants may be a manifestation of a positive adaptive feature due to the influence of the mutation on the photosynthetic apparatus.

As for the chlorophyll-deficient mutant M-81 with the chlorophyll deficiency of *xantha* type, another mechanism of adaptation should be assumed. In the chlorophyll-deficient part of this plant, we observe a decrease in the number of all the test pigments approximately 6-fold compared to the control. Given such a low content of pigments, an increase in the amount of

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carotenoids to the level of chlorophyll *b* certainly can not normalize the process of photosynthesis. From the literature sources it is known that quantitative changes in the composition of pigments arising in response to environmental factors may affect deeper structural and functional modifications of the photosynthetic apparatus (16). In our studies, the *xantha* mutant has a change in the shape of the chloroplasts, which can just be evidence of structural changes in chloroplasts. Changing the shape of chloroplasts in mutant M-81 affects both their linear dimensions and derivatives-volume. It was established that the change in the shape of chloroplasts in the M-81 mutant with *xantha* chlorophyll deficiency persists and at subsequent stages of development - budding and flowering (14). This type of adaptation transformation is also characteristic of cereal chlorophyll-deficient plants, which, according to the authors, exists for individuals with an altered pigment composition, despite the lag in growth and development (21).

The number of photosynthetic pigments and their ratio are important indicators of aging. In addition, according to some authors, the variation in the total ratio of chlorophyll/carotenoids can be a good indicator of stress in plants (22). As our studies show, the ratio of the sum of the two main photosynthetic pigments (chlorophylls *a* and *b*) to the amount of carotenoids is significantly reduced in comparison with the control in the leaves of the chlorophyll-deficient part of the mutants (in the M-80 mutant, almost 2-fold). But in the lower leaves the mutant M-80 (*viridis*) has no significant differences in this ratio, and in the mutant M-81 (*xantha*) in the lower green leaves of the plant this indicator twice exceeds the values of the control variety. This feature can also indicate a different mechanisms of adaptation in the mutants with different type of chlorophyll deficiency.

To obtain a suspension cell culture according to the method of A.T. Mokronosov cuttings are made from different parts of the plant leaf, as a result of which cells from different parts of the leaf blade enter the sample (10). In the process of maceration, some cells are destroyed and it is difficult to say whether the change in the size of cells and chloroplasts is associated with the ecological and physiological parameters of the growing conditions or with the anatomical features of the leaf.

As is known, the mesophyll in the linseed plant is isolateral and has the same structure on the morphologically upper and lower sides of the leaf plate, which is confirmed by our studies (14). Moreover, the columnar cells are comparatively slightly elongated, their length exceeds the width only by 1.5-3.5 times. At the stage of 8-10 leaves there were no differences in the size of the cells of the investigated genotypes (except for the volume in M-80), which may be due to the young age of the investigated leaves.

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Mutual compensation of one pigment to another, a proportional change in the amount of pigments or a change in the shape of chloroplasts may indicate a high plasticity of the photosynthetic apparatus of leaves in linseed plants.

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