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FEATURES OF THE PHOTOSYNTHETIC APPARATUS OF CHLOROPHYLL-DEFICIENT MUTANTS OF VARIOUS TYPES AND THEIR IMPACT ON PRODUCTIVITY TRAITS IN LINSEED

Iryna Poliakova[1], Viktoriia Kolesnyk[1], Olena Boika[1], Viktor Lyakh[1]

[1] Zaporizhzhia National University, 66 Zhukovskogo St, Zaporizhzhia 69600, Ukraine

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ABSTRACT

The unique phenotypic expression and diverse changes in chlorophyll-deficient mutants make them ideal model objects for physiological, biochemical, and genetic studies, particularly in the context of photosynthesis in various cultivated plants.

The paper presents the results of a comparative study of two chlorophyll-deficient mutations of linseed types, *chlorina* and *xantha*, obtained from gamma irradiation of seeds and their initial genotypes. It was found that in the *xantha* type mutation, the disorders affected the morphology of chloroplasts, causing their elongation and, as a result, a change in shape from oval to cylindrical. A decrease in the content of both chlorophylls, especially chlorophyll *b*, was noted. The carotenoid amount also decreased significantly, although it exceeded chlorophyll *b*'s content. Such changes led to a significant decrease in the growth of dry biomass of plants, a decrease in the photosynthetic potential and the net productivity of photosynthesis. It was established that in the *chlorina* type mutation, the disorders caused some elongation of chloroplasts, which did not cause a change in their shape. A decrease in chlorophyll *a* and *b* content by 30 and 45%, respectively, were noted. The content of carotenoids decreased slightly, corresponding to the level of chlorophyll *b*. These changes did not lead to a decrease in the photosynthetic potential and the net productivity of photosynthesis. Based on the mutant sample of *chlorina*, the highly productive variety Zolotistyi was created by the direct selection method, which, in terms of productivity, oil content and yield of oil per hectare, is not inferior to the original line K-7487.

Keywords: linseed, chlorophyll-deficient mutant, pigment, chloroplast, photosynthetic potential, productivity, yield

INTRODUCTION

Researchers face a severe challenge in maintaining plants' high photosynthetic activity, thereby reducing the load on agricultural landscapes. Finding a solution to this problem depends on the presence of highly productive genotypes that use photosynthetically active radiation more efficiently (Zhuykov *et al.*, 2020).

The increase in the inception of photosynthetically active radiation is reached by increasing the leaf index of crops due to elongation of the vegetation terms, the slow-down of the process of ageing of the photosynthetic apparatus in the ontogeny of the leaf, and the widening of the spectrum used for photosynthesis. Such possibilities are connected with the leaves absorbing light. The leaf is a complex system, the optical characteristics of which and the presence of pigments and structure ensure the adsorption of solar energy (Beadle and Long, 1985).

The mechanism of converting physical energy into chemical energy with the help of the "cosmic role" of plants is found to be directly dependent on the peculiarities of the structure of leaf cells and the photosynthetic complex of pigments, in the first place, chlorophylls (Adams and Terashima, 2018). Pigments' absolute content and ratio in any plant species are not constant. It can vary significantly depending on the intensity and quality of light, the structural features of the leaf blade: thickness, the specific surface of the leaf area, and the number of chloroplasts per unit of the leaf area.

The main photosynthetic pigments of the leaf are chlorophylls "a", "b" and carotenoids, which capture the necessary solar energy and protect plants from the harmful by-products of this process. The amount of pigments and their ratio significantly affect plant metabolism (Grjuner, 2018). Chlorophyll *a* is dark green in the leaf, and chlorophyll *b* is yellow-green. Chlorophyll *a* plays a central function in photosystems, while chlorophyll *b* and carotenoids broaden the absorption spectrum and perform a protective function. It is well-known that chlorophylls and carotenoids are the leading factors that affect the possibility of photosynthesis and its intensity. The maximum efficiency of the photosynthetic apparatus of typically developed plants is achieved at a chlorophyll *a*/*b* ratio of 2.5–3.0 (Turmanidze and Dolidze, 2014). Chlorophyll is one of the most critical parameters of the photosynthetic apparatus of plants. According to its content, we can judge the degree of development of the photosynthetic apparatus, the plant's physiological state, and the plant's potential ability to form and accumulate a yield (Kolisnik *et al.*, 2013).

In a comprehensive study of the photosynthetic apparatus, the content of pigments serves as a physiological and biochemical trait. Recently, this indicator has been increasingly used to characterize the functional activity in breeding. For this goal, research is carried out on the state of crops from the point of view of their photosynthetic activity, studying the development of the surface of the leaves. The amount of energy absorbed by sowing, possible primary production of organic substances, and total transpiration depend on leaves' size and spatial structure (Strizhova and Ozhogina, 2005; Zhuykov *et al.*, 2020). One of the most important parameters with the best correlation of yield level is the photosynthetic potential. Management of photosynthesis and increasing its productivity is one of the most effective methods for increasing the productivity of growing plants and an essential method of raising yields (Beadle and Long, 1985).

It is believed that most mutants obtained by experimental mutagenesis result from point recessive mutations. Such mutations are successfully used in various crop plant breeding programs in many countries. Mutation breeding plays an essential role in crop improvement, and mutant varieties have been developed that have improved performance and are resistant to biotic and abiotic stresses (Appa Rao *et al.*, 1984; Arisha *et al.*, 2015; IAEA, 2023; Deshpande and Malode, 2018).

Chlorophyll mutants are used as model objects in physiological and biochemical studies (Wang *et al.*, 2002; Ladygin *et al.*, 2006; Garnik *et al.*, 2015; Lin *et al.*, 2022) and as markers in genetics (Appa Rao *et al.*, 1984; Svetleva, 2005; Wani, 2017). This is because plant chlorophyll formation is the last result of a long chain of biochemical processes involving many loci (Ladygin *et al.*, 2006; Deshpande and Malode, 2018, 2021). Chlorophyll development seems to be controlled by many genes located on several chromosomes, which could be adjacent to centromeres or proximal segments of chromosomes. In addition, chlorophyll-deficient mutants are used as tests to evaluate the genetic action of mutagenic factors because they are the most frequent mutations and are easily identified (Svetleva, 2005; Wu *et al.*, 2007; Wani, 2020). To date, mutant forms with a well-developed chloroplast structure and high productivity have been isolated, from which a conclusion has been drawn about the possibility of influencing the productivity of plants by acting on chloroplasts (Ladygin *et al.*, 2006).

Linseed is an important oil and technical crop in the world (Paul and Kumar, 2019). Therefore, scientific research is actively conducted to study the genetics of this crop and obtain valuable mutant forms for use in breeding practice (Porohovinova, 2019; Deshpande and Malode, 2018). However, the use of chlorophyll mutant forms in breeding programs of the linseed is not described in the scientific literature. The dissertation thesis of E. O. Porokhovinova, which was carried out at the VIPI (Vavilov Institute of Plant Industry), was to investigate the genetic control of four genes *ygp1* (yellow green plant 1), *ygp2* (yellow green plant 2), *zeb1* (zebrine white green plant 1) and *zeb2* (zebrine white green plant 2), which are available in their collection. It was found that these genes are not allelic, and plants carrying these genes are strongly suppressed and have low viability (Porohovinova, 2019).

Our work aimed to compare the pigment complex, structural features of the photosynthetic apparatus, and the influence of these indicators on the change in photosynthetic potential and the formation of yield in two contrasting chlorophyll-defective mutant forms of linseed.

MATERIALS AND METHODS

As materials in the study were used *Linum usitatissimum* subsp. humile Mill. from the collection of the Department of Genetics and Plant Resources of Zaporizhzhia National University (chlorophyll mutants of 2 different types – *chlorina* and *xantha*), cv Tsian (All-Russian Scientific-Research Institute of Oil Crops (ARSRIOC), Krasnodar, RF) and line

K-7487 (Vavilov Institute of Plant Industry (VIPI), St. Petersburg, RF).

Chlorina – The mutant line M-28 was obtained by treating line K-7487 with gamma rays at a dose of 700 Gy. It is yellow-green, beginning at the stage of cotyledon leaves and continuing throughout the growing season.

Xantha – the mutant line M-81 was obtained by treating cv Tsian with gamma rays at 400 Gy. It is bright yellow-green. The brightest individuals are dying, while yellow-green individuals live until the end of the vegetation, with signs of intense depression of the traits and forming a small number of pods.

The initial genotypes cv Tsian and line K-7487 with standard green colour were used as a control.

Setting up experiments and conducting research were carried out in accordance with the methods (Lukomec *et al.*, 2010). All measurements and observations were performed in triplicate. The plants were grown on the experimental plot of the Department of Genetics and Plant Resources of Zaporizhzhia National University in Zaporizhzhia (the North-Western part of the Zaporizhzhia region) in the Southeast of Ukraine. The soil of the plot is ordinary chernozem. Plant descriptions were provided according to generally accepted methods (Lyakh and Poliakova, 2008). The photosynthetic potential was determined by multiplying the average leaf area per 1 ha by the number of days between the first and last records, and the net photosynthetic productivity in certain time intervals was determined by the increase in plant dry weight per unit area during the accounting period (Lukomec *et al.*, 2010). The first measurement was carried out at the beginning of flowering, when 75% of the plants had bloomed. The next measurement was performed after 7 days.

The seed yield was determined separately, completely from each plot Seed oil content was determined using an extraction method according to the State Standard of Technical Conditions (SSTC) of Ukraine No 7577:2014.

For analysis of photosynthetic pigments (chlorophyll *a*, *b* and carotenoids), leaves of mutant lines and their initial forms were taken in the “herringbone” phase. According to the BBCH scale, the “herringbone” phase is Stage 3, namely Stem elongation. During this period, the plants are 28–36 days old and reach a 7–15 cm height.

The amount of the main photosynthetic pigments and the anatomical and morphological features of the plastid apparatus were analyzed simultaneously. The leaves were weighed thoroughly in a mortar with a small amount of $CaCO_3$, quartz sand and 80% acetone. The homogenate was filtered on a folded filter to extract the photosynthetic pigments. Pigments of the photosynthetic apparatus were determined spectrophotometrically using spectrophotometer SF-46 (D&T production) at wavelengths corresponding to the absorption maxima of the pigments in a given solvent: 470, 646, and 663 nm. Control - pure solvent (80% acetone). The calculation was made according to the formula for the extraction of 80% acetone (Barykina *et al.*, 2004; Lichtenthaler and Wellburn, 1983).

To study the morphology of chloroplasts, the selected leaves were fixed in a mixture of Tempera, which retained their color. The leaves were then paraffinized, cross-sectioned on an MPS-2 rotary microtome, and deparaffinized. The resulting preparations were photographed using an XS-3330 LED MICROmed (China) trinocular microscope and an MA88-500 ocular camera at a magnification of 1600 times. Chloroplast sizes were measured by standard methods using an eyepiece micrometer. To characterize the plastid apparatus (determine their shape), the ratio of the length and width of chloroplasts was calculated (Lyakh *et al.*, 2016).

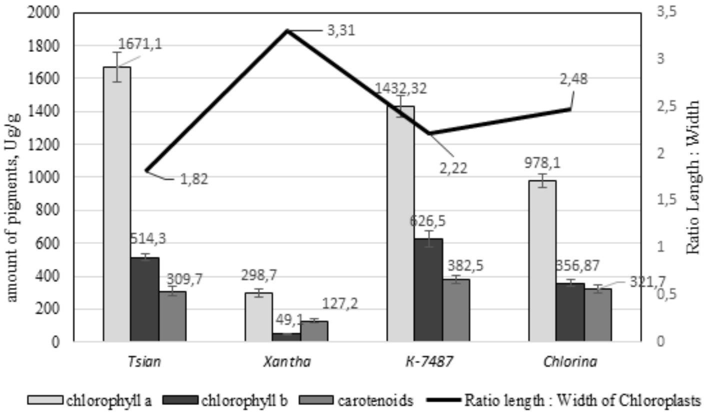
The received data were statistically processed on a computer using Microsoft Office Excel and the modern statistics package “STATISTICA 10.” The obtained data were subjected to mathematical processing by dispersion and correlation analyses (Littl and Hills, 1981; Dospekhov, 1989).

RESULTS AND DISCUSSION

We chose the mutant forms of chlorina and xantha because they differ greatly in phenotypic manifestation and viability. Our collection of chlorophyll-deficient mutant samples also contains other mutant lines obtained by treating linseed seeds with various mutagens. In addition, it was chlorina and xantha mutations that had a higher frequency than other forms of chlorophyll mutations. The same trend was noted by other researchers on beans (Svetleva, 2005), lentils (Wani, 2017), cowpea (Wani, 2020), as well as Indian scientists in linseed (Deshpande and Malode, 2018).

To find out exactly what changes in the photosynthetic apparatus occurred in our induced chlorophyll-deficient mutant types *chlorina* and *xantha,* we studied their pigment composition compared to their initial genotypes. As is known, plastid pigments are the main components of the photosynthetic apparatus of plants responsible for the absorption of light energy and its transformation into the energy of chemical bonds. The size and state of the pool of photosynthetic pigments in the leaf largely determine the physiological state of plants at different stages of ontogeny under various environmental conditions, and plant adaptation to the level of illumination is carried out due to dynamic variation in the content and ratio of pigments (Turmanidze and Dolidze, 2014).

Our research revealed that the total content of chlorophylls in both mutants is significantly reduced (Fig. 1). Thus, the reduction of this trait in the *chlorina* type was about 35%, and in the *xantha* type - 84%. If we consider the content of chlorophyll *b* alone, we can see even more significant changes. The decrease in the content of this pigment in the *chlorina* type is 43%, and in the *xantha* type, it is



1: *The content of photosynthetic pigments and the ratio of length: width of chloroplasts in chlorophyll-deficient mutants of linseed and initial genotypes with the normal green color of plants*

even more than 90%. It is known from the literature that existing chlorophyll-deficient mutants can be divided into three groups: mutants for chlorophyll *a*, mutants for chlorophyll *b*, and mutants for both types of chlorophylls. Mutants of the second group are more common (Ladygin *et al.*, 2006). Since we observed changes in two types of chlorophylls, we referred these mutants to mutants for both types of chlorophylls or to the third group.

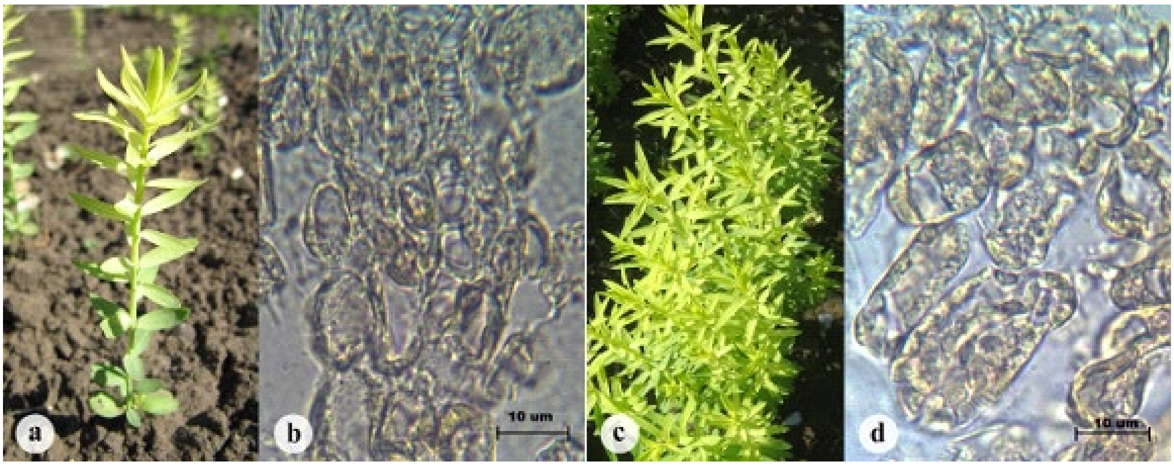
As for carotenoids, in the chlorophyll-deficient mutants studied by us, their ratio changes compared to chlorophyll *b*. Thus, in control green plants, the amount of carotenoids is about 60% compared to the content of chlorophyll *b*. Whereas, in the *chlorina* type, the amount of carotenoids almost does not change compared to the control, and the ratio for chlorophyll *b* approaches 90%. Even more significant changes were noted in *xantha*. In this type, the content of carotenoids is even more than chlorophyll *b* by 150% or 2.6 times. It is this change in the ratio of chlorophylls a and b, which causes the dark green and yellow-green colour of plants, as well as the increase in the role of carotenoids, and determines, in our opinion, the yellow-green and bright yellow-green colour of the leaves and plants of these mutants. Probably, carotenoids, in this case, play a protective role, protecting chlorophylls from photodegradation, and also partially compensating for their absorption capacity. We think that this can be one of the ways of adaptation of chlorophyll-deficient plants to environmental conditions. The change in the Chl/Car and Chl a/b ratios indicates a rearrangement of the light-harvesting complexes of photosystems and an increase in the role of carotenoids as additional light-harvesters in the blue-violet region of the solar spectrum. Usually, in normal green leaves, the ratio (Chl a + b)/Car is = 3–8 (Turmanidze and Dolidze, 2014). In our case, in normal plants, this ratio was 7.1 for Tsian and 5.4 for K-7487. The ratio is significantly lower in chlorophyll-deficient mutants: 4.2 for the *chlorina* type and 2.7 for the *xantha* type. Such chromatic adaptations probably expand the possibilities of light absorption and improve the plasticity of mutant plants.

Determining the ratio of chlorophylls to carotenoids is important in characterizing the operation of the photosynthetic apparatus. The content of chlorophylls and carotenoids is determined by the genotype of the variety, the phase of development, the degree of its plasticity and resistance to environmental factors. It is an informative trait indicating the degree of adaptability of plants to various adverse environmental factors.

British scientists obtained data close to our observations in the study of light-harvesting and photosynthetic characteristics of the chlorophyll-deficient mutant of cowpea (*Vigna unguilata*), resulting from a mutation of one nuclear gene. They found a decrease in the total chlorophyll content by 40% and concluded that a significant loss of chlorophyll in the leaves of the mutant does not necessarily have a detrimental effect on photosynthesis and plant productivity (Dimah *et al.*, 1994). In addition to changes in the pigment composition of the investigated mutant forms, we also detected changes in the morphology of plants' photosynthetic apparatus (Fig. 2).

To study the peculiarities of the structure of the linseed photosynthetic apparatus, we have developed and patented a method for determining the morphology of the plastid apparatus of plants (Lyakh *et al.*, 2016). When conducting a comparative analysis of the anatomical structure of leaves of chlorophyll-deficient mutants and their original forms, we found that these mutants are also characterized by changes in the structure of their plastid apparatus relative to control green plants.

We discovered changes in the morphology of such photosynthetic cell structures as chloroplasts. As can be seen from the diagram (Fig. 1) according to the ratio of length: width (l:d), the chloroplasts of



xantha *chlorina*

2: *Phenotypic expression of chlorophyll-deficient mutations of linseed types xantha (a) and chlorina (c) and morphological features of their chloroplasts (b, d, respectively)*

normal green plants have a ratio of these indicators in the range of 1.8–2.22, corresponding to the shape of an oval. In the mutant lines, elongation is noted. In *chlorina*, the elongation is small, compared to the original line K-7487. The calculated indicator of 2.48 corresponds to the ratio of 2.5, which has the shape of an oval. Therefore, it can be observed in the photo that the shape of the chloroplasts of the *chlorina* mutant sample did not differ from the control (Fig. 2).

However, significant changes have taken place in the xantha type. The ratio is 3.31, which, in our opinion, indicates a significant deformation of chloroplasts. According to the ratio of linear indicators, we note a change in the shape of chloroplasts from oval to cylindrical. This can also be observed in the photo. According to our observations, the chloroplasts of the *xantha* type are narrow and elongated, so they differ in shape from the control and are the narrowest among the studied genotypes.

Thus, we concluded that in the mutant line of the *chlorina* type, the content of chlorophylls *a* and *b* decreased because of the mutation. At the same time, the content of carotenoids remained at the control level, and chloroplasts almost did not change their shape. And the *xantha* type line was characterized by a change in the shape of chloroplasts, which became elongated, a significant decrease in the total amount of all pigments, especially chlorophyll *b*. At the same time, as a compensatory effect, the content of carotenoids exceeded the content of chlorophyll *b*.

Chinese scientists also used a similar approach to describe changes in the ultrastructure of the chloroplast in the study of chlorophyll-deficient wheat mutants. They found that the leaf morphology of the Mt6172 mutant was different from that of other registered wheat mutants and, on this basis, identified it as a new mutant form (Guo *et al.*, 2012).

In the study of the chlorophyll mutant *Brassica napus* L. with yellow leaves, a significantly lower content of chlorophyll *a*, *b*, and carotenoids was noted than in the wild type. Therefore, the net efficiency of photosynthesis, stomatal conductance, and transpiration rate were significantly reduced. In addition, the mutant had a sparse distribution of chloroplasts, granule walls were reduced, and the shape was extremely irregular (Lin *et al.*, 2022). These data are also consistent with our results (Fig. 1 and 2).

The chlorophyll-deficient mutant lines chlorina and *xantha* we studied are very contrasting regarding viability and productivity. The *xantha* mutant line is characterized by a strong delay in growth and development and very low productivity. The chlorina mutant line, despite the yellow colour of the entire plant during the entire growing season, produces productivity (yield and oil content) at the same level as the control. Therefore, we used them and their initial forms to research the impact of changing the photosynthetic apparatus on the formation of productivity. This approach was successfully used to study the processes of photosynthesis and the structures directly involved in it, and in other cultures (Ladygin *et al.*, 2006; Dimah *et al.*, 1994; Lin *et al.*, 2022).

The main traits that determine the accumulation rate of organic mass are the growth rates of the leaf surface area and the intensity and productivity of photosynthesis. The photosynthetic assimilation leaf apparatus is characterized by optimal dimensions, formation speed and duration of its functioning. The full use of such a renewable and ecologically clean intensification factor as solar radiation depends on spatial orientation as an optical system of chlorophyll saturation, photosynthesis intensity, productivity, and other components of photosynthetic activity (Hopkins, 2006).

During our research, it was found that at the "herringbone-budding" stage, the studied genotypes differ significantly in terms of photosynthetic potential. A difference was established between

I: *Formation of the photosynthetic potential and net productivity of photosynthesis of different genotypes of linseed (2018–2020)*

Photosynthetic potential, t day ha^{-1}		Increase in dry biomass for the period, t ha^{-1}		Net productivity of photosynthesis per day, g/m^2	
“herringbone-budding”	“budding-flowering”	“herringbone-budding”	“budding-flowering”	“herringbone-budding”	“budding-flowering”
Tsian (green)					
347.5	420.3	1.13	2.56	3.88	7.30
Xantha (yellow-white)					
118.1	150.9	0.34	0.53	3.37	4.16
K-7487 (dark green)					
365.2	436.5	1.20	2.72	3.92	7.50
Chlorina (yellow-green)					
375.4	432.1	1.29	2.84	4.09	7.89
LSD $_{05}$ 4.09	11.0	0.01	0.16	0.09	0.24

LSD – a least significant difference

the two original samples of Tsian and K-7487 and between the original genotypes and their mutant forms. At the next stage, “budding-flowering”, the trend persists, however, significant differences were established only between the Tsian variety and the *xantha* mutant line (Tab. I).

The trait “increase in dry biomass for the period” revealed a similar pattern. At an earlier stage of ontogenesis, there is a significant difference between all studied genotypes. This difference is levelled off at the “budding-flowering” stage, and significant changes are established only between the *xantha* mutant line and its initial form, the Tsian variety.

Since organic substances are formed in the process of photosynthesis from carbon dioxide and water due to absorbed solar energy, the productivity of crops depends on their ability to absorb radiation and convert it into the energy of chemical bonds for further use in metabolic processes. The highest values of the net productivity of photosynthesis were found in the *chlorina* mutant sample. In both phases of the study, it significantly outperforms its original form, the Tsian variety, and another *xantha* mutant line. That is why, in our opinion, this line should be considered as a donor for improving the indicators of photosynthetic activity for linseed.

In contrast to *chlorina*, the *xantha* mutant line at both stages of the study had significantly lower indicators for all the investigated characteristics and is characterized by a decrease in photosynthetic activity indicators. These data coincide with the results of other researchers, which indicate a significantly lower intensity of photosynthesis in chlorophyll mutants than the intensity of photosynthesis in green plants, reduced viability, and depression (Ladygin *et al.*, 2006). In the study of the white tissue of the striped plant and the albino wheat plant, a complete loss of photosynthetic function was revealed. As a result, the photosynthesis of the studied Mt135 mutant was severely reduced and limited. This change in the photosynthetic process decreased plant height and spike length (Zhao *et al.*, 2011).

In the study of Russian scientists with populations of perennial grasses, significant differences were noted in terms of seed weight per plant between plants with suppressed chlorophyll deficiency and the initial type. As chlorophyll deficiency (viridis–xantha—albina) increased, the weight of seeds per plant decreased from 30 to 48% (Lebedeva *et al.*, 2012).

Viability, survival, and fertility are the most critical traits of plant fitness at different stages of ontogeny within a generation. The most significant indicator of viability is reproductive potential, which characterizes the ability of a plant to produce enough seeds to reproduce offspring. In addition, most leaf colour mutants have reduced photosynthesis efficiency, which seriously negatively impacts crop growth and economic output.

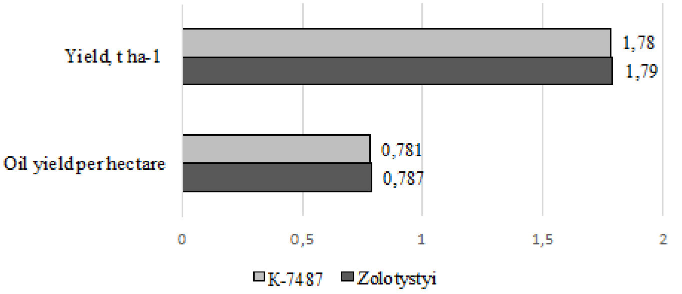
The yield of linseed, like any plant, is determined mainly by the size and productivity of the assimilation apparatus. We created a high-yielding linseed variety, Zolotystyi, by direct selection of a chlorina-type chlorophyll-deficient mutation. After studying the complex of economic and valuable traits, this line was included in the breeding programs of the laboratory of linseed breeding and genetics of the Institute of Oil Crops of the National Academy of Agrarian Sciences of Ukraine (Zaporizhzhia), where it was evaluated in various breeding nurseries and submitted for registration under the name variety Zolotystyi. In addition, it successfully passed the variety test under No 06020.

According to the results of our three-year study, the Zolotystyi variety has no significant differences from its original line K-7487, both in terms of the oil content in the seeds and the yield level. As seen from the data in Tab. II, the Zolotystyi variety yields 1.42–2.15 t ha^{-1}, and the yield of the K-7487 line

II: *Economically valuable traits of the linseed variety Zolotystyi (mutant of the chlorina type) in comparison with the initial form (2018–2020)*

Year	Genotype	Yield, t ha^{-1}	Oil content, %	Oil yield per hectare, t ha^{-1}	LSD	
					Yield	Oil yield
2018	K-7487	1.39	48.6	0.601	0.08	0.4
	Zolotystyi	1.42	48.4	0.612		
2019	K-7487	1.70	48.8	0,738	0.11	0.5
	Zolotystyi	1.79	49.2	0.784		
2020	K-7487	2.25	50.1	1.003	0.12	0.5
	Zolotystyi	2.15	50.5	0.966		

LSD – a least significant difference



3: *Comparison of the average productivity and oil yield per hectare in the variety of mutant origin Zolotystyi and its original line (2018–2020)*

is 1.39–2.25 t ha^{-1}. Both genotypes produced the highest yield in 2020, which amounted to 2.25 t ha^{-1} for K-7487 and 2.15 t ha^{-1} for Zolotystyi.

As seen from the data in Tab. II, the oil content of the Zolotystyi variety also has values close to the initial line. This critical trait in different years was 48.4–50.5% for the Zolotystyi variety and 46.8–50.1% for the K-7487 line. In 2020, both genotypes showed the highest values of oil content in seeds, namely 50.5 and 50.1%, respectively.

The «oil yield per hectare» trait determines the efficiency of growing oil crops. This indicator is a derivative of the productivity and oil content trait. As can be seen from Fig. 3, on average, over the years of research, it was 0.787 t ha^{-1} in the Zolotystyi variety and 0.781 t ha^{-1} in its initial line K-7487. Such differences are insignificant and within the experiment's margin of error.

Based on the presented results, we concluded that the *chlorina* type mutation we obtained did not disrupt the structure and functions of the photosynthetic apparatus, and plants can provide a high productivity level. However, it is essential to note that the Zolotystyi variety is characterized by an evident morphological trait "yellow-green colour of the plant during the entire growing season." In our opinion, this genotype is a valuable genetic source for further genetic and breeding work. Therefore, it should be involved in breeding programs as a donor of a complex of significant economic and valuable traits, namely high oiliness, high productivity, and the marker-trait "yellow-green colour of the plant during the growing season."

In studies on fennel mutagenesis, a group of Indian scientists also selected chlorophyll-deficient mutants that showed changes in flowering time, number of primary branches, number of umbels per plant, number of seeds per umbel, and days to maturity. Some yielded more than the original form (Verma *et al.*, 2018).

Therefore, the work on obtaining chlorophyll-deficient lines of linseed, studying them, and involving them in breeding is a promising direction that will help deepen knowledge about the functioning of the photosynthetic apparatus of linseed plants and establish ways to improve its work.

CONCLUSION

A comprehensive comparative study of two chlorophyll-deficient mutations of the *chlorina* and *xantha* types and their initial forms established several differences in the pigment complex, the structure of the plastid apparatus, the photosynthetic potential, and the net productivity of photosynthesis.

It was revealed that the *xantha* type mutation affected the structure of chloroplasts, causing their elongation and changing shape from oval to cylindrical. It led to a sharp decrease in the content of both chlorophylls, especially chlorophyll *b*. The amount of carotenoids also decreased but exceeded the content of chlorophyll *b*. Such significant changes led to a substantial decrease in the net productivity of photosynthesis.

It was found that in the *chlorina* type mutation, the disorders caused a slight elongation of chloroplasts, but without changing their shape. A decrease in the content of all pigments was noted, which is revealed in the chlorophyll deficiency of the whole plant, but it was less than in the *xantha* type mutation. These changes did not lead to a decrease in photosynthetic potential, net productivity of photosynthesis, accumulation of oil in seeds and formation of yield.

Based on the chlorophyll-deficient mutation *chlorina* type, by direct selection, a highly productive variety of linseed Zolotystyi was created, which is not inferior to the original form in terms of yield, oil content and oil yield per unit area.

REFERENCES

ADAMS III., W. W. and TERASHIMA, I. 2018. The Leaf: A Platform for Performing Photosynthesis. *Springer International Publishing AG, part of Springer Nature*. 575 p. https://doi.org/10.1007/978-3-319-93594-2

ARISHA, M. H., SHAH, S. N. M., GONG, Z. H., JING, H., LI, C. and ZHANG, H. X. 2015. Ethyl methane sulfonate induced mutations in M_2 generation and physiological variations in M_1 generation of peppers (*Capsicum annuum* L.). *Frontiers in Plant Science*. 6, 399. https://doi.org/10.3389/fpls.2015.00399

BARYKINA, R. P., VESELOVA, T. D. and DEVJATOV, A. G. 2004. *Handbook of botanical microtechnology. Fundamentals and methods.* [in Russian: Справочник по ботанической микротехнике. Основы и методы.] Moscow: Moscow University Press. 312 p.

BEADLE, C. L. and LONG, S. P. 1985. Photosynthesis: is it limiting to biomass production? *Biomass*. 8(2), 119–168. https://doi.org/10.1016/0144-4565(85)90022-8

DESHPANDE, A. S. and MALODE, S. N. 2018. Induced Mutation Studies in *Linum usitatissimum* Var. PKV NL – 260 With Reference To Mitotic Abnormalities. In: *Multidisciplinary International Conference on Green Earth: A Pararomic View*, 197–200.

DIMAH, Z. H., BEMARD, G. and NEIL, R. B. 1994. The consequences of chlorophyll deficiency for photosynthetic light use efficiency in a single nuclear gene mutation of cowpea. *Photosynthesis Research*. 42, 17–25.

DOSPEKHOV, B. A. 1989. *Field study methods*. [in Russian: Методика полевого опыта]. Moscow: Kolos. 416 p.

GARNIK, E. Y., DEEVA, D. V., BELKOV, V. I., TARASENKO, V. I. and KONSTANTINOV, Y. M. 2015. Effects of Light Intensity on Development and Chlorophyll Content in the Arabidopsis Mutant Plants with Defects in Photosynthesis [in Russian]. *Journal of Stress Physiology & Biochemistry*. 11(4), 58–67.

GRJUNER, L. A. 2018. Quantity and ratio of photosynthetic pigments in blackberry leaves [in Russian: Количество и соотношение фотосинтетических пигментов в листьях ежевики. Современное садоводство]. *Contemporary horticulture*. 3, 74–80. https://doi.org/10.24411/2312-6701-2018-10311

GUO, HUI-JUN, ZHAO, HONG-BING, ZHAO, LIN-SHU, GU, JIA-YU, ZHAO, SHI-RONG, LI, JUN-HUI, LIU, QING CHANG and LIU, LU-XIANG. 2012. Characterization of a Novel Chlorophyll-Deficient Mutant Mt6172 in Wheat. *Journal of Integrative Agriculture*. 11(6), 888–897. https://doi.org/10.1016/S2095-3119(12)60079-3

HOPKINS, W. 2006. *Photosynthesis and respiration*. Chelsea House, 168 p.

KOLISNIK, J. S., KISLICHENKO, V. S. and KUZNICOVA, V. J. 2013. Pigments of buckwheat (*Capsella bursa-pastoris*) grass [in Ukrainian: Пігменти трави грициків звичайних (*Capsella bursa-pastoris*)]. *Pharmaceutical journal*. 1: 75–77.

LADYGIN, V. G., SEMENOVA, G. A. and SHEGAJ, I. D. 2006. Structural and functional organization of chloroplasts in the *xantha*-702 mutant of cotton *Gossypium hirsutum* L. [in Russian: Структурно-функциональная организация хлоропластов у мутанта *xantha*-702 хлопчатника *Gossypium hirsutum* L.]. *Cytology*. 48(7), 537–553. ISSN 0041-3771

LEBEDEVA, O. N., NIKOLAEVSKAJA, T. S., TITOV, A. F. and FEDORENKO, O. M. 2012. *Biological features of northern populations of perennial grasses. Genetic load and survival* [in Russian: Биологические особенности северных популяций многолетних злаков. Генетический груз и выживаемость.]. Petrozavodsk: Karelian Scientific Center of the Russian Academy of Sciences. 261 p.

APPA, RAO, S., MENGESHA, MELAK, H. and RAJAGOPAL, REDDY C. 1984. Characteristics and inheritance of viable chlorophyll mutants in *Pennisetum americanum* (L.) *Leeke. Indian J. Bot.* 7(1), 1–5.

LICHTENTHALER, H. K. and WELLBURN, A. R. 1983. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochem. Soc. Trans.* 11(5), 591–592. https://doi.org/10.1042/bst0110591

LIN, N., GAO, Y., ZHOU, Q., PING, X., LI, J., LIU, L. and YIN, J. 2022. Genetic mapping and physiological analysis of chlorophyll-deficient mutant in *Brassica napus* L. *BMC Plant Biol.* 18; 22(1), 244. https://doi.org/10.1186/s12870-022-03630-9

LITTL, T. and HILLS, F. 1981. *Agricultural experimental work. Planning and analysis.* Moscow: Kolos. 320 p.

LUKOMEC, V. M., TISHKOV, N. M., BARANOV, V. F., PIVEN, V. T., UGO, TORO KORREA, SHULJAK, I. I. 2010. *Methodology for conducting field agrotechnical experiments with oilseeds* [in Russian: Методика проведения полевых агротехнических опытов с масличными культурами]. Krasnodar: VNIIMK. 327 p

LYAKH, V. O., YARANTSEVA, V. V., LEVCHUK, H. M. and POLIAKOVA, I. O. 2016. Inventor; State higher educational institution “Zaporizhsky National University” of the Ministry of Education and Science of Ukraine., assignee. *Method for determining morphology of plant plastid apparatus* [in Ukrainian: Спосіб визначення морфології пластидного апарату рослин.]. Ukrainian patent UA 106114. https://uapatents.com/6-106144-sposib-viznachennya-morfologi-plastidnogo-aparatu-roslin.html

LYAKH, V. O. and POLIAKOVA, I. O. 2008. *Breeding of linseed. Methodical recommendations* [in Ukrainian: Селекція льону олійного. Методичні рекомендації.]. Zaporizhzhia: Zaporizhzhia National University. 37 p.

WANI, MOHAMMAD RAFIQ. 2020. Characterization of chlorophyll deficient mutants in mungbean (*Vigna radiata* (L.) Wilczek). *Bangladesh J. Bot*. 49(4), 1013–1019. https://doi.org/10.3329/bjb.v49i4.52532

WANI, MOHAMMAD RAFIQ. 2017. Induced Chlorophyll Mutations, Comparative Mutagenic Effectiveness and Efficiency of Chemical Mutagens in Lentils (*Lens culinaris* Medik). *Asian J. Plant Sci*. 16(4), 221–226. https://doi.org/10.3923/ajps.2017.221.226

INTERNATIONAL ATOMIC ENERGY AGENCY (IAEA). 2023. Mutant variety database. 2019–2022 [online]. https://mvd.iaea.org [Accessed 2022-04-07].

PAUL, S. and KUMAR, N. 2019. Selection strategy and estimation of interrelationships for improvement of seed yield and other related traits in linseed (*Linum usitatissimum* L.). *Indian Journal of Plant Genetic Resources*. 32(1), 28–35. https://doi.org/10.5958/0976-1926.2019.00004.4

POROHOVINOVA, E. A. 2019. Genetic collection of flax (*Linum usitatissimum* L.): creation, analysis and prospects for use [in Russian: Генетическая коллекция льна (*Linum usitatissimum* L.): создание, анализ и перспективы использования]. Dissertation thesis. St. Petersburg.

STRIZHOVA, F. M. and OZHOGINA, L. V. 2005. Formation of leaf surface area by varieties of spring wheat [in Russian: Формирование площади листовой поверхности сортами яровой пшеницы]. *Bulletin of the Altai State Agrarian University*. 4(20), 16–19.

SVETLEVA, D. L. 2004. Induction of chlorophyll mutants in common bean under the action of chemical mutagens ENU and EMS. *Journal Central European Agriculture*. 5(2), 85–90.

TURMANIDZE, N. M. and DOLIDZE, K. G. 2014. The results of studying the dynamics of the content of plastid pigments in the leaves of a tea plant [in Russian: Результаты изучения динамики содержания пластидных пигментов в листьях чайного растения]. *Basic Research*. 9–9, 2009–2012.

VERMA, A. K., DHANASEKAR, P., CHOUDHARY, Sharda, MEENA, R. D., LAL, Gopal. 2018. Estimation of induced variability in M2 generation of fennel (*Foeniculum vulgare* Mill.). *Journal of Pharmacognosy and Phytochemistry*. 7(1), 430–436.

WANG, J., GOSZTOLA, D., RUFFLE, S. V., HEMANN, C., SEIBERT, M., MICHAEL, R., WASIELEWSKI, R. H., GUSTAFSON, T. L. and SAYRE, R. T. 2002. Functional asymmetry of photosystem II D1 and D2 peripheral chlorophyll mutants of *Chlamydomonas reinhardtii*. *PNAS* 99(6): 4091–4096. https://doi.org/10.1073pnas.0620568999

WU, Z., ZHANG, X,. HE, B., DIAO, L., SHENG, S. and WANG, J. 2007. Chlorophyll deficient rice mutant with impaired chlorophyllide esterification in chlorophyll biosynthesis. *Plant Physiology*. 145, 29–40. https://doi.org/10.1104/pp.107.100321

ZHAO, HONG-BING, GUO, HUI-JUN, ZHAO, LIN-SHU, GU, JIA-YU, ZHAO, SHI-RONG, LI, JUN-HUI, LIU, LU-XIANG. 2011. Agronomic Traits and Photosynthetic Characteristics of Chlorophyll-Deficient Wheat Mutant Induced by Spaceflight Environment. *Acta Agron Sin.* 37(1), 119–126. https://doi.org/10.3724/SP.J.1006.2011.00119

ZHUYKOV, O., BURDIUG, O., USHKARENKO, V., LAVRENKO, S. and LAVRENKO, N. 2020. Photosynthetic activity and productivity of sunflower hybrids in organic and traditional cultivation technologies. *AgroLife Scientific Journal*. 9(1), 374–381.

Contact information

Iryna Poliakova: https://orcid.org/0000-0003-3181-3111
Olena Boika: olena.boika.ua@gmail.com, https://orcid.org/0000-0002-1680-6477 (corresponding author)
Viktor Lyakh: https://orcid.org/0000-0002-7385-3157