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Features of the Pigment Complex of Winter Wheat Plants Depending on the Method of Fertilisation

Abstract. To maximise the yield of winter wheat, it is necessary to further optimise modern cultivation technologies to adapt them to changing environmental conditions. One of the aspects of plant adaptation to unfavourable abiotic factors is the active functioning of the photosynthetic apparatus, which depends on the number of nutrients applied. This paper investigates the effect of fertilisation on the state of the pigment complex of winter wheat plants in the Southern Steppe of Ukraine. Two varieties of winter wheat were selected for the study: Shestopalivka and Mason. The scheme of the experiment included the application of fertilisers during sowing (K_0; K_{12}) and foliar processing with various tank mixtures (urea; urea + magnesium sulphate; urea + magnesium sulphate + potassium monophosphate). The pigment content was determined by grinding fresh leaves of winter wheat with further addition of a solvent in the form of acetone. The pigments were measured using a spectrophotometer. The results of the studies showed that before foliar fertilisation, the content of chlorophyll *a* and carotenoids was higher in the leaves of plants of the Shestopalivka variety. At the same time, the content of chlorophyll b was higher for Mason plants by 17%, which may be a consequence of the adaptation of plants of this variety to a lack of light. A decrease in the pigment content in the leaves

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of plants of all experimental variants caused by the active growth of the photosynthesising surface and a decrease in the total dry matter mass was observed on day 3 after the foliar fertilisation. There was no significant difference between varieties in the content of photosynthetic pigments during this period. On the 10^{th} day after foliar spraying, an increase in the content of chlorophyll a and b was observed for both studied varieties, which may be the result of the adaptation of the photosynthetic apparatus of winter wheat plants to lighting conditions. Foliar fertilisation of winter wheat plants with a tank mixture of urea with magnesium sulphate and potassium monophosphate contributed to a further increase in the content of chlorophyll a by 12-23% and chlorophyll b by 5-37%, depending on the variety compared to the control. The results of the conducted research indicate the high efficiency of the complex application of nitrogen-phosphorus-potassium fertilisers for foliar spraying of winter wheat plants at the stage of BBCH 31 both on the background of pre-sowing potassium fertilizers and without it

Keywords: *Triticum aestivum*, foliar fertilisation, chlorophylls a and b, carotenoids, LHC

RELEVANCE

Every year the technology of growing such an important food crop as winter wheat is further improved. However, there are still a number of factors that play an important role in shaping the quality and size of the future harvest. One of them is the rational application of mineral fertilisers, which, when optimally combined, increase the yield and improve the quality of winter wheat grain (Kalytka & Zolotukhyna, 2013).

An important component of the process of forming vegetative and generative organs of a crop is the photosynthetic activity of crops (Cherenkov *et al.*, 2015). Maintaining the active functioning of the photosynthetic apparatus of winter wheat leaves during the tube phase, which is the period of intensive plant growth and development and maximum absorption of moisture and nutrients, is one of the decisive factors in the formation of a high-quality crop yield (Gharanjik *et al.*, 2019). During this period, it is important to increase the supply of nitrogen to plants through foliar fertilisation, which is mainly carried out with fertilisers such as urea. This agronomic technique is widely used in farming, as this fertiliser is easily absorbed and combined with other foliar treatments (Sokolovska-Serhiienko *et al.*, 2016).

Another important factor contributing to high yields is the optimal supply of phosphorus and potassium nutrition. The application of potash fertilisers during sowing has a significant impact on plants during the growing season, which further affects the future harvest (Kudriavytska & Karabach, 2020). Meanwhile, there has been a growing interest in the foliar application of phosphorus-potassium fertilisers in the early tapering phase (Huliaieva *et al.*, 2015).

The active passage of photosynthesis serves to better supply nitrogen from the roots to the aboveground part of the plant. A photosynthetic apparatus is a volumetric reservoir for storing organic forms of nitrogen. That is why its efficiency determines the future yield potential of plants (Sheheda *et al.*, 2018), is the basis of their productivity, and largely depends on the content and ratio of pigments in plant leaves. Chlorophylls *a* and *b* are indicators of the physiological state of plants, the functioning and activity of which are indicators of the potential ability of plants to form a crop. Another essential component of pigment systems is carotenoids, which are light-emitting pigments that protect chlorophyll from destruction during oxidative stress. The main functions performed by carotenoids are antioxidant, antennal, photoprotective, and structural.

The content of photosynthetic pigments (chlorophyll a and b and carotenoids) shows the physiological state of plants, their photosynthetic potential and the measurement of capacity, modelling, and prediction of the efficiency of the applied cultivation technology (Vagusevičienė *et al.*, 2012). The number of pigments in plants depends on a number of factors: genetic properties of the variety, stage of growth and development, environmental influences, and sowing density (Juchnevičienė & Vagusevičienė, 2015). The plant growing environment affects the mechanisms of pigment activity. Thus, fertiliser deficiency can lead to a decrease in pigment content in plant leaves (Smolikova & Medvedev, 2015). At the same time, improvements in the photosynthetic characteristics of the flag leaf, which is formed at the end of the tube exit phase, contribute to a high yield (Noor *et al.*, 2021)

ANALYSIS OF RECENT RESEARCH AND PUBLICATIONS

Feeding with nitrogen fertilisers during the phase of entering the tube significantly increases the content of chlorophyll a and b and carotenoids. Previously, it was established that the synthesis of chlorophyll depends on mineral nutrition. Such a measure affects the dynamics of leaf surface formation and the degree of leaf surface, which is reflected in the total leaf surface, photosynthetic potential and net photosynthetic productivity. The highest concentration of chlorophylls accumulates in the leaves shortly before flowering (Vagusevičienė *et al.*, 2012).

According to the results of research by Aistė Juchnevičienė and Ilona Vagusevičienė, it was found that fertilisation with nitrogen fertilisers stimulates the accumulation of photosynthetic pigments and prolongs the period of active photosynthesis. Chlorophyll is extremely sensitive to changes in the nitrogen content in the soil because most of the nitrogen is part of the pigments. A properly selected fertiliser system, including foliar fertilisation, delays the degradation of photosynthetic pigments and prolongs the period of active photosynthesis and ensures active transport of assimilates to the seeds, thereby determining the yield (Juchnevičienė & Vagusevičienė, 2015).

Foliar fertilisation is becoming a more common practice in agriculture to increase yields. It can effectively make up for nutrient deficiencies and can be used as the main method of providing plants with the necessary micronutrients (Jankowski *et al.*, 2016).

The results of research by I.B. Kovalyshyn and V.V. Shevchenko reveal that foliar treatment with phosphorus-containing fertilisers preserves the activity of the photosynthetic apparatus and slows down the ageing process during the reproductive development of winter wheat plants (Kovalyshyn & Shevchenko, 2020).

The purpose of the study is to determine the effect of the fertilisation method on the state of the pigment complex of winter wheat plants in the Southern Steppe of Ukraine.

MATERIALS AND METHODS

The study was conducted at leading enterprises in the Melitopol district of Zaporizhzhia oblast in 2019-2021. Two varieties of winter wheat were used: Shestopalivka, the most common variety in the south-eastern region of Ukraine (Bilousova, 2018), classified as a physiologically dual-nature variety, and Mason, a Canadian transgenic winter wheat variety developed in 2016 using nanotechnology to transform wheat DNA cells. The two-factor experiment included determining the effect of pre-sowing potassium fertilisation (K_0; K_{12}) and foliar treatment of plants with different tank mixtures in the phase of the beginning of the tube emergence: urea (N (control)); urea + magnesium sulphate (N+Mg); urea + magnesium sulphate + potassium monophosphate (N+Mg+PK) on the content of the main photosynthetic pigments. The consumption rate of urea is 10 kg/ha, magnesium sulfate – 2 kg/ha, and potassium monophosphate – 1 kg/ha.

To determine the pigment content, fresh leaves of winter wheat were crushed with scissors and a 0.1-0.2 g sample was ground in a porcelain mortar with $CaCO_3$. 5 ml of acetone solvent was added and stirred. The resulting suspension was then filtered into a dry measuring cone through a filter. 10 ml of acetone was re-added to the mortar, rinsed and filtered. The filter was washed with acetone until the pigments were completely removed. The resulting extract was poured into a 50 ml flask and brought up to the mark with a solvent and stored in a dark place before determining the pigments. The pigment content was determined using a 2800 UV/VIS SPECTROPHOTOMETER at wavelengths of 440.5, 644, and 662 nm (Hrytsaienko et al, 2003). The obtained data were statistically analysed using Microsoft Excel.

RESULTS

The chlorophyll content of winter wheat leaves affects the process of solar energy absorption by plants. At the same time, carotenoids are important pigments that characterise the photosynthetic apparatus of plants and transfer the energy of the absorbed light quantum to chlorophyll for photochemical work and protect chloroplasts from photooxidation. As demonstrated by the results of the studies (Table 1), the analysed winter wheat varieties differed in pigment content and their ratio.

Table 1. The state of the pigment complex of winter wheat plants at stage BBSN 31 before foliar fertilisation, the average for 2020-2021

Cultivar (factor A)	Pre-sowing fertilisation (factor B)	Foliar fertilisation (factor C)	Pigment content, mg/g of dry matter			(chl.*a*)/(chl.*b*)	chl/car
			chlorophyll *a*	chlorophyll *b*	carotenoids		
Shestopalivka	K_0	N (k)	4.94±0.76	1.46±0.17	1.91±0.30	3.38	3.35
		N±Mg	4.92±0.66	1.49±0.30	1.88±0.21	3.30	3.41
		N±Mg±PK	4.98±0.50	1.52±0.12	1.88±0.26	3.28	3.46
	K_{12}	N (k)	4.20±0.99	1.64±0.40	1.74±0.33	2.56	3.36
		N±Mg	4.19±0.94	1.64±0.45	1.64±0.24	2.55	3.55
		N±Mg+PK	4.21±0.95	1.69±0.58	1.67±0.33	2.49	3.53

Table 1, Continued

Cultivar (factor A)	Pre-sowing fertilisation (factor B)	Foliar fertilisation (factor C)	Pigment content, mg/g of dry matter			(chl.*a*)/(chl.*b*)	chl/car
			chlorophyll *a*	chlorophyll *b*	carotenoids		
Mason	K_0	N (k)	4.22 ± 0.91	1.71 ± 0.46	1.61 ± 0.32	2.47	3.68
		N ± Mg	4.33 ± 0.89	1.78 ± 0.62	1.70 ± 0.37	2.43	3.59
		N ± Mg ± PK	4.28 ± 0.94	1.77 ± 0.32	1.69 ± 0.23	2.42	3.58
	K_{12}	N (k)	3.87 ± 1.22	1.64 ± 0.41	1.50 ± 0.44	2.36	3.67
		N ± Mg	3.92 ± 1.26	1.72 ± 0.60	1.45 ± 0.37	2.28	3.89
		N ± Mg ± PK	3.83 ± 1.27	1.70 ± 0.44	1.48 ± 0.26	2.25	3.74

Thus, before the foliar fertilisation, the content of chlorophyll *a* in the leaves of winter wheat plants of Shestopalivka variety was 15% higher, and carotenoids – 16% higher compared to Mason variety. At the same time, the content of chlorophyll *b* was higher in Mason variety by 17%, which may be due to the adaptation of plants of this variety to a lack of light (Klipakova et al, 2021). The ratio Chl *a* / Chl *b* confirms this conclusion.

Pre-sowing potassium fertilisation led to a decrease in the concentration of pigments at the stage of BBCH 31 in the leaves of both varieties of winter wheat, which can be explained by the active growth of the leaf surface area and their corresponding growth dilution.

On day 3 after the foliar fertilisation (Table 2), a decrease in the pigment content in the leaves of plants of all experimental variants was observed, which was again due to the active growth of the photosynthetic surface and a decrease in the total dry matter mass. At the same time, there was no significant difference between varieties in the content of photosynthetic pigments during this period.

Table 2. The state of the pigment complex of winter wheat plants at stage BBSN 31 on the 3rd day after foliar fertilisation, the average for 2020-2021

Cultivar (factor A)	Pre-sowing fertilisation (factor B)	Foliar fertilisation (factor C)	Pigment content, mg/g of dry matter			(chl.*a*)/(chl.*b*)	chl/car
			chlorophyll *a*	chlorophyll *b*	carotenoids		
Shestopalivka	K_0	N (k)	3.67 ± 0.92	1.79 ± 0.49	1.50 ± 0.87	2.05	3.64
		N ± Mg	3.99 ± 0.83	1.89 ± 0.73	1.79 ± 0.25	2.11	3.28
		N ± Mg ± PK	4.29 ± 0.86	1.96 ± 0.46	1.55 ± 0.24	2.19	4.03

Table 2, Continued

Cultivar (factor A)	Pre-sowing fertilisation (factor B)	Foliar fertilisation (factor C)	Pigment content, mg/g of dry matter			(chl.*a*)/ (chl.*b*)	chl/car
			chlorophyll *a*	chlorophyll *b*	carotenoids		
Shestopalivka	K_{12}	N (k)	3.81±0.83	1.75±0.29	1.93±0.12	2.18	2.88
		N±Mg	4.08±0.78	1.78±0.54	1.76±0.04	2.29	3.33
		N±Mg±PK	4.19±0.24	1.80±0.50	1.74±0.25	2.33	3.44
Mason	K_0	N (k)	3.50±0.57	1.74±0.41	1.51±0.28	2.01	3.47
		N±Mg	3.68±0.68	2.01±0.52	1.80±0.21	1.83	3.16
		N±Mg±PK	4.19±0.75	2.05±0.31	1.47±0.16	2.04	4.24
	K_{12}	N (k)	3.61± 0.74	1.87±0.74	1.55±0.22	1.93	3.54
		N±Mg	3.99±0.70	1.96±0.38	1.69±0.20	2.04	3.52
		N±Mg±PK	4.11±0.54	1.98±0.33	1.60±0.23	2.08	3.81

Notably, on the 3rd day after fertilisation, as opposed to the previous period, there was no significant difference in pigment content between the variants with pre-sowing potash fertilisation and those without such agricultural practices.

Foliar fertilisation of plants contributed to the growth of both chlorophylls and carotenoids in the leaves of plants of both studied varieties. Moreover, the highest efficiency of this agricultural practice was observed in the variants of complex application of urea with magnesium sulfate and potassium monophosphate both on the background of pre-sowing fertilisation with potassium fertilisers and without it.

During this period, the Chl. *a* / Chl. *b* ratio decreased by 6-39% compared to the previous stage, depending on the experimental variant, resulting from an increase in the content of chlorophyll *b* in response to an increase in leaf surface area and deterioration of lighting conditions. The complex application of foliar fertilisers (N + Mg + PK) against the background of pre-sowing K_{12} fertilisation contributed to a more stable performance of the photosynthetic apparatus of plants of both studied varieties, as a result of which the indicated chlorophyll ratio differed only by 6-8% compared to the previous period.

On day 10 after the foliar fertilisation treatment, an increase in chlorophyll content was observed (Table 3), which indicates a gradual adaptation of the photosynthetic apparatus of winter wheat plants to the habitat conditions.

Moreover, the Shestopalivka variety showed an increase in both chlorophyll *a* and chlorophyll *b* content by 6-22% and 3-7%, respectively, depending on the treatment option, compared to the previous stage.

At the same time, the Mason variety was characterised by an increase in the content of only chlorophyll *b* by 9-32% compared to day 3 after fertilisation.

Table 3. The state of the pigment complex of winter wheat plants at stage BBSN 31 on the 10th day after foliar fertilisation, the average for 2020-2021

Cultivar (factor A)	Pre-sowing fertilisation (factor B)	Foliar fertilisation (factor C)	Pigment content, mg/g of dry matter			(chl.*a*)/(chl.*b*)	chl/car
			chlorophyll *a*	chlorophyll *b*	carotenoids		
Shestopalivka	K_0	N (k)	4.47±0.14	1.96±0.10	1.48±0.01	2.28	4.34
		N±Mg	4.80±0.49	1.94±0.17	1.94±0.10	2.47	3.47
		N±Mg±PK	5.08±0.32	2.06±0.04	1.56±0.14	2.47	4.58
	K_{12}	N (k)	4.30±0.14	1.92±0.11	1.74±0.09	2.24	3.57
		N±Mg	4.32±0.25	1.94±0.15	1.63±0.30	2.23	3.84
		N±Mg±PK	4.80±0.07	2.11±0.09	1.59±0.05	2.27	4.35
Mason	K_0	N (k)	3.56±0.22	1.90±0.24	1.48±0.04	1.87	3.69
		N±Mg	3.80±0.14	2.40±0.28	1.65±0.15	1.58	3.76
		N±Mg±PK	4.38±0.29	2.60±0.13	1.45±0.06	1.68	4.81
	K_{12}	N (k)	3.79±0.67	2.08±0.27	1.51±0.27	1.82	3.89
		N±Mg	4.05±0.90	2.42±0.39	1.77±0.27	1.67	3.66
		N±Mg±PK	4.37±0.67	2.62±0.22	1.59±0.30	1.67	4.40

Foliar fertilisation of winter wheat plants with phosphorus-potassium fertilisers (N + Mg + PK) contributed to a further increase in chlorophyll *a* content by 12-23% and chlorophyll *b* by 5-37% depending on the variety, as compared to the control. Meanwhile, the highest efficiency of this agricultural practice was achieved with the Mason variety both with and without potash fertilisers.

The content of carotenoids remained largely the same as in the previous analysed period, which also confirms the stable performance of the leaf apparatus at this stage of plant development.

This stage of development was characterised by an increase in the ratio of chlorophylls to carotenoids (Chl./car.) – from 2.88-4.24 on day 3 after fertilisation to 3.47-4.81 on day 10, which indicates the predominance of green pigments in the photosynthetic apparatus of winter wheat plants and its active functioning.

The light-harvesting complex (LHC) characterises the number of chlorophylls engaged in the transfer of absorbed energy to the pigment-protein complex and plays an important role in the regulation of the light stage of photosynthesis. As demonstrated by the data obtained, foliar fertilisation of winter wheat plants at the stage of BBCH 31 contributed to an increase in the number of pigments entering the LHC on day 3 with a gradual decrease in their content on day 10 (Fig. 1). This surge in the activity of sunlight absorption by pigments is most likely attributed to an increase in the activity of the plant enzyme system as a result of foliar fertiliser (Bilousova et al, 2019).

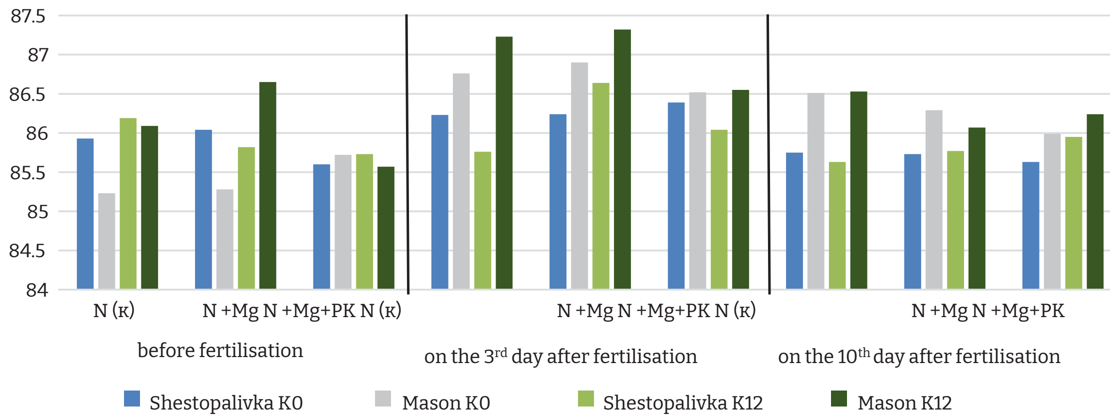

Figure 1. The activity of the light-harvesting complex of winter wheat plants depending on the studied factors, the average for 2020-2021, %

The highest activity of LHC at all analysed stages was observed for Mason plants, which may be the result of adaptation to deteriorating lighting conditions due to active growth of leaf surface area and is consistent with the information presented above.

CONCLUSIONS

The results of the conducted research indicate the high efficiency of the complex application of nitrogen-phosphorus-potassium fertilisers for foliar fertilisation of winter wheat plants at the stage of BBCH 31 both on the background of pre-sowing application of potassium fertilisers and without it. Thus, this agricultural technique resulted in a 10-17% increase in chlorophyll *a* and a 3-10% increase in chlorophyll b content for Shestopalivka variety compared to the control. At the same time, for Mason, this increase was 14-23% and 6-37%, respectively.

The adjustment of the plant nutrition system at different stages of growth and development helps to increase the content of photosynthetic pigments in the leaves of winter wheat plants, which in the future affects plant yield and the quality of the grain.

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Особливості пігментного комплексу рослин пшениці озимої залежно від способу удобрення

Анотація. Для отримання максимальної врожайності озимої пшениці необхідна подальша оптимізація сучасних технологій вирощування з метою їх адаптації до мінливих умов навколишнього середовища. Одним із аспектів адаптації рослин до несприятливих абіотичних факторів є активне функціонування фотосинтетичного апарату, яке залежить від кількості внесених поживних речовин. У роботі досліджено вплив удобрення на стан пігментного комплексу рослин озимої пшениці в умовах Південного Степу України. Для дослідження було обрано два сорти озимої пшениці: Шестопалівка та Мейсон. Схема досліду включала внесення добрив під час сівби (K_0; K_{12}) та позакореневі обробки різними баковими сумішами (карбамід; карбамід + сульфат магнію; карбамід + сульфат магнію + монофосфат калію). Вміст пігментів визначали шляхом подрібнення свіжого листя озимої пшениці з подальшим додаванням розчинника у вигляді ацетону. Вміст пігментів вимірювали за допомогою спектрофотометра. Результати досліджень показали, що до позакореневого підживлення вміст хлорофілу *a* та каротиноїдів був вищим у листках рослин сорту Шестопалівка. Водночас вміст хлорофілу *b* був вищим у рослин сорту Мейсон на 17%, що може бути наслідком адаптації рослин цього сорту до нестачі світла. На 3-й день після позакореневого підживлення спостерігалося зниження вмісту пігментів у листках рослин усіх дослідних варіантів, що пов'язано з активним ростом фотосинтезуючої поверхні та зменшенням загальної маси сухої речовини. Суттєвої різниці між сортами за вмістом фотосинтетичних пігментів у цей період не спостерігалося. На 10-ту добу після позакореневого підживлення спостерігали збільшення вмісту хлорофілу a і b для обох досліджуваних сортів, що може бути наслідком адаптації фотосинтетичного апарату рослин пшениці озимої до умов освітлення. Позакореневе підживлення рослин пшениці озимої баковою сумішшю карбаміду з сульфатом магнію та монофосфатом калію сприяло подальшому підвищенню вмісту хлорофілу a на 12-23% та хлорофілу b на 5-37% залежно від сорту порівняно з контролем. Результати проведених досліджень свідчать про високу ефективність комплексного застосування азотно-фосфорно-калійних добрив для позакореневого обприскування рослин пшениці озимої у фазі BBCH 31 як на фоні передпосівного внесення калійних добрив, так і без нього

Ключові слова: *Triticum aestivum*, позакореневе підживлення, хлорофіли a і b, каротиноїди, ЛГК