Accumulation of pigments in wheat leaves under influence of macronutrients

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Abstract. On natural acidic soil, statistically significant relationships between wheat yield and pigment content in leaves were established (for *Chl a* r = 0.910...0,949; *Chl b* = 0.925…0,967; for *carotenoids* in 2017 = 0.887;). Nitrogen fertilizer promoted an increase in pigment content in leaves (*Chl a* and *Chl b* - by 38…43%, carotenoids - by 16…22%). At phosphorus application, the content of chlorophylls increased by 44…83%, carotenoids - by 20…37%. The content of mobile phosphorus in the arable layer of natural acidic soil is statistically significantly associated with the content of *Chl b* (r = 0.738 and 0.793 for 2017 and 2020, respectively) and the part of pigments included in light-harvesting complexes (r = 0.799 and 0.829, respectively). In 2020, statistically significant associations of mobile phosphorus content in soil and weight ratios of *Chl a* / *Chl b* (r = -0.815) and (*Chl a* + *Chl b*) / carotenoids (r = 0.840) were noted. Soil liming reduced the influence of phosphorus on the pigment complex of leaves: against a natural background, the increase in *Chl* content was 27…40%, against a limed background – 27…29%; for *Chl b* - on the contrary, liming increased its content by 93…108%, while on a natural background - only by 63…84%. On both soil backgrounds, the addition of phosphorus reduced the carotenoid content in wheat leaves (on a natural background - by 7…9%, on limed - by 17…23%).

1 Introduction

Phosphorus (P) is the second macroelement after nitrogen that limits plant growth and development. It is involved in numerous cellular processes: activation of various enzymes, transfer of energy and regulatory signals, regulation of metabolic processes, etc. [1, 2]. However, unlike nitrogen, phosphorus cannot be obtained with the participation of microorganisms, so the amount of phosphorus available for plants is finite [3]. In acidic soils around the world, the bioavailability of phosphorus to plants is quite low due to the high levels of iron and aluminum [4], and its application is necessary to maintain modern crop production [5].

Wheat (*Triticum aestivum* L.) is one of the most important food crops in the world; according to FAO, in 2019 it was produced more than 765 million tons (http://www.fao.org/faostat/en/#data/QC). It is known that wheat production is highly

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dependent on the amount of phosphorus fertilizer application, therefore, compared to other main crops, when cultivating wheat, it is necessary to contribute more phosphorus per unit area [6]. Therefore, improving the efficiency of phosphorus fertilizers in wheat cultivation is one of the main goals of sustainable agricultural production [7].

Chlorophylls and carotenoids are the most important structural and functional components of the photosynthetic apparatus of grain leaves, which provides the creation of organic matter of plants (i.e. their productivity). In case of change of growth conditions, for example, under influence of different level of application of mineral fertilizers or presence of stress factors, quantitative parameters of pigment complex of leaves change adapting to new environment conditions [8, 9]. At the same time, such physiological parameters of flag leaf as chlorophyll content are considered reliable indicators of grain productivity [10] since chlorophyll content positively correlates with photosynthetic ability [11]. The relationship between phosphorus and chlorophylls is not fully understood. The review [12] expressed the view that there was still no consensus in the conclusions of the work on these relationships due to differences in sampling methods and analytical methods. The effect of phosphorus on the content of chlorophyll can be indirect and complex, since phosphorus can indirectly influence or interfere with the absorption of other nutrients [13], which in turn affects the synthesis and accumulation of chlorophyll in plants. According to these authors, plants that live on low-fertile, phosphorus-poor soils will experience minimal effect or even be depressed by phosphorus fertilizers. Previously, Wu et al. [14] showed that no significant changes in chlorophyll A and B, the total content of chlorophyll and carotenoids were found in phosphorus stress; and phosphorus stress generally had no effect on photosynthesis. On the other hand, an increase in phosphorus concentration under acidic conditions of hydroponic growth of *Salvia chamelaeagnea* K. Bergius significantly increased the synthesis of chlorophyll [15].

Our previous studies have shown that in the upper horizons of natural sod-podzolic soils of the Kirov region (Russian Federation), the aluminum content can reach 250-300 mg/kg of soil [16], which strongly affects the bioavailability of phosphorus to plants. Ninety-three percent of the phosphate stock of the arable layer is represented by iron (72%), calcium (11%) and aluminum (10%) phosphates. Annual application of high doses of superphosphate (from 50 to 200 kg/ha) can provide in such conditions the content of mobile phosphorus from 191 to 285 mg/kg (without liming), and when adding lime according to one hydrolytic acidity - from 222 to 376 mg/kg [16]. Therefore, the aim of the presented studies is to assess the effect of increasing levels of soil mobile phosphorus and lime on the qualitative and quantitative parameters of the pigment complex of flag leaves of spring wheat (*Triticum aestivum* L.).

2 Materials and methods

Research was carried out on spring wheat plants as part of the long stationary experiment of the Falenki breeding station - a branch of the Federal Agricultural Research Center of the North-East (the central agro-climatic zone of the Kirov region of the Russian Federation). The type of soil of the experimental site is sod-podzolic middle-loamy, on cover loams. The experiment is putted down in quadruple replications on plots with a total area of 40.25 m^2 each. In the study, the influence of two factors was investigated - the soil acidity and the level of mineral nutrition. In the case of first factor, two soil backgrounds were used: a natural acidic soil with a pH of 3.68...3.93 and a soil background, on which lime was added according to hydrolytic acidity (24 kg per plot or 5 962 kg/ha), until the pH of 5.26...5.61 was reached. To assess the effect of the second factor in both soil backgrounds (acidic and close to neutral), fertilizers were applied to create the necessary levels of soil phosphorus according to the scheme: in 2008...2014 – 1st variant - control without fertilizers; 2nd variant - application of nitrogen fertilizer in amount of N90; 3-6th variants - on the background of application of N90K90, phosphorus was added at rates P50, P100, P150 and P200. In 2015...2020 the scheme was slightly changed: in variants 3 - 6 the same amount of fertilizers was applied (N90P50K90). Moreover, according to chemical analyses of the soil, variants 3-6 differed in the content of mobile phosphorus (the real amount is indicated in tab. 3, 4).

Next spring wheat cultivars were grown in study: Iren' (originator - Ural Agricultural Research Institute, Yekaterinburg, Russian Federation), admitted to production since 2000 and Bazhenka (originator - FARC of the North-East, Kirov, Russian Federation), admitted to production since 2011. Both cultivars are early with good technological qualities of grain characteristic of valuable wheat.

To assess the qualitative and quantitative composition of the pigment complex of wheat flag leaves at the flowering stage samples were taken from twenty individual plants of each plot on all test variants. In acetone extracts of leaf samples, pigment content was determined at wavelengths of 470, 644.8 and 661.6 nm on a spectrophotometer "UVmini-1240" manufactured by Shimadzu Corporation (Japan) in triplicate analytical replications according to procedure [17]. By the same procedure, chlorophylls *a* and *b* (*Chl a*, *Chl b*) and carotenoids (*Car*) were calculated per dry leaf matter. The amount of pigments included in the light-harvesting complexes (LHC) was calculated by the formula [18]:

$$Chl \text{ in LHC} = [(1.2\ Chl\ b + Chl\ b) / (Chl\ a + Chl\ b)] \quad (1)$$

Weather conditions developing during the growing period had a direct impact on the physiological processes of growth and development of spring wheat plants. Samples were taken on July 17 in 2017 and July 9 in 2020. The temperature regime of the 2017 growing period, with the exception of the second decade of July, was below the climatic norm (tab. 1). Due to cold and wet weather in May, wheat sowing was carried out 2 weeks later than many years. In June, insufficient precipitation was noted, in July (before the sampling of plants) - excessive humidification.

Table 1. Meteorological conditions of growing period, s. Falenki.

Month	Air temperature, °C					Precipitations, mm				
	on ten-day periods			per month	± to normal *	on ten-day periods			per month	% to normal *
	I	II	III			I	II	III		
2017										
May	6.6	7.2	8.6	7.5	-3.1	28.8	17.7	12.4	58.9	134
June	11.6	16.0	14.3	14.0	-2.1	22.0	18.9	17.7	58.6	79
July	13.9	20.0	18.1	17.3	-1.0	58.8	47.9	52.2	158.9	237
2020										
May	13.5	11.0	11.8	12.1	+1.5	1.0	13.2	50.9	65.1	148
June	15.8	15.5	11.5	14.3	-1.8	24.4	5.8	15.6	45.8	62
July	20.5	22.1	17.9	20.2	+1.9	16.4	11.4	56.3	84.1	126

Note: * - climatic normal of temperature / precipitation - the average long-term value calculated in accordance with the requirements of the WMO for a 30-year period (1981... 2010)

The weather conditions of the growing season ("seedlings – tillering - leaf tube formation - earing") in 2020 were unstable in both temperature and precipitations. The dry and hot weather of the first ten-day period of July contributed to the onset of the flowering stage earlier than in 2017. The hydrothermal coefficient of the "germination-flowering" period in 2017 was 1.8, in 2020 - 1.6.

The methods of descriptive statistics, ANOVA and correlation analyses presented in the table editor of Microsoft Office Excel 2016 were used to statistically process the obtained data. The tables show the arithmetic mean and their standard errors. The statistical significance of the differences between the investigated variants, as well as the degree of influence of factors on plant development indicators and the state of the pigment complex, was estimated at $p \leq 0.05$.

3 Results and discussion

Analysis of data on the yield of spring wheat plants in 2017 and 2020 (tab. 2) showed that an increase in the level of mobile phosphorus (from P50 to P200) did not lead to a statistically significant change in the investigated parameter in both tested cultivars. In other words, the creation in the arable soil layer of an increase in the concentration of mobile phosphorus by 25-30% compared to the natural background (from 60-70 to 75-80 mg/kg of soil in 2017 and from 120 to 160 mg/kg in 2020) creates optimal conditions for the growth and development of wheat plants.

Table 2. Yield of spring wheat cultivars at different levels of soil phosphorus.

Variant	Iren' (2017)	Bazhenka (2020)
No lime		
No fertilizers	1.05±0.19	2.12±0.34
N90	1.90±0.02	2.93±0.86
N90P50K90	1.98±0.27	3.86±0.13
N90P100K90	2.00±0.86	3.69±0.26
N90P150K90	2.50±0.14	3.78±0.08
N90P200K90	2.61±0.38	3.28±0.15
Lime application		
No fertilizers	1.43±0.01	2.94±0.15
N90	2.22±0.14	3.21±0.53
N90P50K90	2.79±0.40	3.52±0.09
N90P100K90	1.51±0.08*	3.77±0.27
N90P150K90	1.70±0.40*	3.97±0.24
N90P200K90	2.51±0.13	2.74±0.61

Note: * yield was lowered due to high lodging of plants

At the same time, it can be noted that the application of nitrogen fertilizers on the natural background led to the same increase in wheat yields as the liming without fertilizers (2017) or even higher (2020).

The application of lime in most study variants of the experiment did not have a statistically significant effect on wheat yields in either 2017 or 2020. This may be due to the fact that the content of mobile phosphorus in the soil of the study site was also not affected by the addition of lime (see tab. 3, 4). The data obtained by us to some extent contrasts with the literary data on the positive effect of lime on wheat growth and development [19], since it increases the content of phosphorus available to plants [20]. However, data from other researchers show that under conditions of acidic soils, the application of high doses of lime can lead to fixation of phosphorus with calcium, which will reduce its bioavailability [21]. Another explanation for this result may be the rather low genotypic efficiency of phosphorus absorption by the wheat cultivars used by us: it is known that in many countries of the world in recent decades intensive breeding work has begun on breeding cultivars of crops with high responsiveness to the application of phosphorus fertilizers [22], however, for wheat cultivars of domestic breeding of such information is not enough.

The parameters of the development of the pigment complex of wheat flag leaves depending on the addition of lime material and the amount of available phosphorus in the soil of the experimental site of the Falenki breeding station are shown in tab. 3 and 4.

Table 3. Parameters of development of pigment complex in flag leaves of spring wheat cv. Iren' (mg per g of dry matter), 2017.

Variant	P_2O_5 mg per kg of soil	*Chl a*	*Chl b*	*Car*	*Chl a / Chl b*	*Chl a+b / Car*	*Chl* in LHC, %
No lime							
No fertilizers	58±8	6.45±0.24	3.72±0.19	2.30±0.09	1.73±0.06	4.41±0.13	80.68±0.33
N90	60±1	9.05±0.27	5.33±0.25	2.66±0.11	1.71±0.05	5.41±0.15	81.40±0.95
N90P50K90	75±11	10.09±0.58	6.00±0.49	2.86±0.11	1.69±0.05	5.64±0.30	81.82±1.47
N90P100K90	108±12	10.03±0.18	5.79±0.21	2.92±0.04	1.75±0.03	5.15±0.14	81.22±0.90
N90P150K90	132±6	10.37±0.34	6.26±0.25	2.83±0.19	1.65±0.04	5.87±0.12	82.92±0.36
N90P200K90	141±3	10.87±0.30	6.58±0.16	2.97±0.09	1.66±0.05	5.89±0.21	82.75±0.73
average	96±15	9.48±0.65	5.61±0.42	2.76±0.10	1.70±0.02	5.40±0.23	81.80±0.36
Coefficient of variation, %	38.1	16.9	18.2	9.0	2.3	10.4	1.1
Correlation with P content		0.75	0.77*	0.73	-0.60	0.67	0.82*
Lime application							
No fertilizers	68±6	5.67±0.24	3.19±0.16	2.23±0.09	1.78±0.05	3.97±0.11	79.18±1.35
N90	66±12	8.99±0.34	5.13±0.20	2.80±0.18	1.76±0.06	5.06±0.20	79.78±1.54
N90P50K90	80±13	9.26±0.12	5.40±0.15	2.76±0.19	1.73±0.08	5.31±0.18	80.72±0.74
N90P100K90	107±11	10.87±0.50	6.79±0.30	2.83±0.06	1.62±0.08	6.72±0.25	84.36±2.40
N90P150K90	150±12	10.27±0.19	6.00±0.10	3.00±0.17	1.72±0.07	5.42±0.17	81.04±0.89
N90P200K90	156±6	11.06±0.27	6.68±0.32	3.15±0.03	1.66±0.08	5.65±0.18	82.69±1.73
average	105±16	9.35±0.81	5.53±0.54	2.80±0.13	1.71±0.02	5.36±0.36	81.30±0.78
Coefficient of variation, %	38.6	21.3	24.0	11.2	3.6	16.6	2.4
Correlation with P content		0.71	0.70	0.75	-0.60	0.47	0.56

Note: * - coefficient of correlation is statistically significant at $p \leq 0.05$

Table 4. Parameters of development of pigment complex in flag leaves of spring wheat cv. Bazhenka (mg per g of dry matter), 2020.

Variant	P_2O_5 mg per kg of soil	*Chl a*	*Chl b*	Car	*Chl a / Chl b*	*Chl a+b /* Car	*Chl* in LHC, %
No lime							
No fertilizers	101±15	6.29±0.14	2.86±0.19	2.50±0.12	2.20±0.07	3.67±0.22	68.78±1.55
N90	104±22	6.63±0.02	3.50±0.17	2.31±0.11	1.91±0.08	4.41±0.21	75.75±1.49
N90P50K90	172±10	8.78±0.11	5.13±0.18	2.54±0.19	1.73±0.14	5.56±0.35	80.96±2.86
N90P100K90	251±19	8.42±0.28	5.28±0.14	2.27±0.13	1.60±0.07	5.73±0.18	84.83±1.61
N90P150K90	282±10	7.91±0.19	4.65±0.09	2.32±0.10	1.72±0.11	5.44±0.21	81.23±1.49
N90P200K90	237±35	8.09±0.46	4.86±0.53	2.30±0.04	1.70±0.13	5.63±0.38	81.98±2.94
average	191±32	7.68±0.41	4.39±0.40	2.37±0.05	1.81±0.09	5.07±0.34	78.92±2.36
Coefficient of variation, %	42.9	12.8	22.0	5.7	12.2	17.5	7.5
Correlation with P content		0.72	0.79*	-0.52	-0.82*	0.84*	0.83*

Lime application							
No fertilizers	135±17	6.03±0.27	2.96±0.33	2.18±0.13	2.08±0.14	4.16±0.36	71.89±2.30
N90	135±17	5.85±0.47	3.13±0.29	2.07±0.16	1.89±0.05	4.33±0.20	76.39±1.30
N90P50K90	167±15	8.12±0.30	5.96±0.12	1.98±0.19	1.38±0.06	7.41±0.35	93.01±1.32
N90P100K90	202±13	8.14±0.23	5.87±0.25	1.97±0.13	1.32±0.07	7.14±0.24	92.09±2.70
N90P150K90	290±10	7.96±0.20	5.53±0.37	2.00±0.15	1.46±0.15	6.82±0.44	89.91±2.28
N90P200K90	264±9	8.14±0.21	6.01±0.23	1.92±0.27	1.36±0.07	7.52±0.41	93.57±1.60
average	199±27	7.37±0.45	4.91±0.59	2.02±0.04	1.59±0.13	6.23±0.64	86.14±3.87
Coefficient of variation, %	37.4	14.8	29.1	9.7	19.8	26.2	11.3
Correlation with P content		0.72	0.70	-0.68	-0.67	0.70	0.69

Note: * - coefficient of correlation is statistically significant at $p \leq 0.05$

The application of nitrogen fertilizer (N90) reduced the negative effect of natural acidic soil on the content of pigments in leaves of plants of the cv. Iren' to a lesser extent (an increase in chlorophyll content - by 38-43%, carotenoids - by 16-22%) than the phosphorus application (chlorophylls - by 44-83%, carotenoids - by 20-37%). These observations do not contradict the literary data, which indicate that there are genotypic differences between wheat cultivars in the level of chlorophyll synthesis at the application of phosphorus and nitrogen fertilizers: on some genotypes, the greater effectiveness of phosphorus use is shown, on others - nitrogen [23]. However, neither the application of phosphorus nor the liming nor their co-action had a statistically significant effect on the proportion of pigments in the LHC, i.e., did not affect the structural status of the leaf pigment complex.

By correlation analysis, it was revealed that for cv. Iren' plants, the pigment content in the leaves had a significant strong relationship with yield only on a natural unlimed background: the coefficients of paired correlations were 0.949 for *Chl a*; 0,967 for *Chl b*; 0.887 for carotenoids. On a limed soil background, correlation coefficients were statistically insignificant (0.260 to 0.452).

The content of mobile phosphorus in the soil is statistically related to the content of *Chl b* ($r = 0.769$) and the proportion of pigments in LHC ($r = 0.823$) on a lime-free background. At the background of the application of lime, statistically significant correlations between the studied parameters were not revealed.

For Bazhenka plants, the increase in the content at the use of nitrogen fertilizer was more manifested for *Chl b* (by 22%), the content of *Chl a* increased by 5% only and there were no significant differences in the carotenoid content (in the variant of natural acidic sod-podzolic soil). At the same time, statistically significant alterations occurred in the structure of the pigment complex: the proportion of chlorophylls involved in the capture of sunlight (i.e., their content in the composition of antenna complexes of photosystems) increased by 10%, respectively, their content in the reaction centers of photosystems decreased. Liming leveled the differences in the content of pigments in the leaves, but the restructuring of the structure of the complex itself still occurred in the same direction as on natural soil - the content of chlorophylls in the composition of light-harvesting antenna complexes increased by 6%.

The application of phosphorus on a natural acidic background was more effective in terms of *Chl a* accumulation parameters in Bazhenka plants (an increase of 27-40%) than on a limed background (by 27-29%); for *Chl b* - on the contrary, liming increased its content by 93-108%, while on a natural background - only by 63-84%; however, liming significantly reduced the carotenoid content in the leaves by 17-23%, while on a natural background - by 7-9%. Liming reduced the carotenoid content of leaves in all variants of the experiment (by 11-22%); *Chl a* content was either at the level of unlimed background (variants 5 and 6) or slightly lower (by 3-12%); the content of *Chl b* increased (by 11-24%) with simultaneous exposure to phosphorus, or decreased (by 11%) at nitrogen application;

with no nitrogen and phosphorus added, the *Chl b* content remained substantially unchanged.

As the results of the correlation analysis showed, the content of chlorophylls in the leaves of Bazhenka plants had a significant strong relationship with yield only on an unlimed background: the coefficients of paired correlations were 0.910 for *Chl a*; 0,925 for *Chl b*; for carotenoids the association was insignificant. The content of mobile phosphorus in the soil is statistically associated with the content of *Chl b* (r = 0.793), the ratio of chlorophylls *a*/*b* (r = -0.815), green/yellow pigments (r = 0.840) and the proportion of pigments in LHC (r = 0.829) on a lime-free background. As in the case of wheat cv. Iren' at the liming background, statistically significant correlations between the studied parameters of the pigment complex of flag leaves, on the one hand, and plant yields or the content of mobile phosphorus in the soil, on the other hand, were not detected.

In the course of agronomic studies of the effect of phosphorus on the growth and development of cereal crops, most works evaluate the elements of the yield structure or the efficiency of phosphorus absorption by plants [24, 25]. At the same time, there are quite few works in which the reaction of the pigment complex of wheat leaves to the application of various doses of phosphorus fertilizers would be evaluated. From this point of view, the presented work contributes to clarifying the mechanisms of the influence of phosphorus nutrition conditions on plant yields in terms of changes in the quantitative and qualitative status of the pigment complex of spring wheat leaves, as the basis for assimilation activities of plants and the production of high economic yields.

4 Conclusions

The results of many years of research show that the change in the pH of the soil solution from 3.68...3.93 to 5.26...5.61 by adding lime did not have a statistically significant effect on the content of mobile phosphorus in the soil of experimental sites (for all variants). This option of agricultural measures in most experimental variants also did not lead to a logical change in the level of spring wheat yields. The change in the level of mobile phosphorus by the application of increasing doses of superphosphate (from 50 to 200 kg per ha) did not statistically affect the yield of the two wheat cultivar tested. On the other hand, the effect of liming of natural acidic soil on the yield of spring soft wheat is quantitatively comparable to the effect of application of nitrogen fertilizer alone at a dose of 90 kg per ha.

Application of nitrogen fertilizer on naturally acidic soil led to an increase in pigments in flag leaves (*Chl a* and *Chl b* - by 38-43%, carotenoids - by 16-22%). The application of phosphorus had a similar effect (the content of chlorophylls increased by 44-83%, carotenoids - by 20-37%).

At a natural acidic soil background, statistically significant relationships between the yield level of spring soft wheat and the pigment content in flag leaves of plants appeared (in 2017 for *Chl a* 0.949; *Chl b* 0,967; for carotenoids 0.887; in 2020 for *Chl a* 0.910; *Chl b* 0,925; for carotenoids, the association was insignificant).

The content of mobile phosphorus in the arable layer of natural acidic soil was statistically significantly associated with the content of *Chl b* (r = 0.738 and 0.793 for 2017 and 2020, respectively) and the proportions of pigments included in the LHC (r = 0.799 and 0.829, respectively). For the cv. Bazhenka (2020), statistically significant associations were also noted between the content of mobile phosphorus in the soil and such structural parameters of the pigment complex of flag leaves as the weight ratios *Chl a* / *Chl b* (r = -0.815) and (*Chl a* + *Chl b*) / carotenoids (r = 0.840). In liming soil variants, these relationships became statistically insignificant.

The liming of acidic soil modified the effect of phosphorus on the pigment complex of wheat leaves: at a natural background, the increase in the content of *Chl a* was 27-40%, at a limed background - 27-29%; for *Chl b* - on the contrary, liming increased its content by 93-108%, while on a natural background - only by 63-84%. On both soil backgrounds, the application of phosphorus reduced the carotenoid content in wheat leaves (on a natural background - by 7-9%, on liming background - by 17-23%).

Liming reduced the carotenoid content in leaves in all variants of the experiment (by 11-22%); *Chl a* content was either at the level of unlimed background (variants 5 and 6) or slightly lower (by 3-12%); *Chl b* content increased (by 11-24%) with simultaneous exposure to phosphorus, or decreased (by 11%) at nitrogen application.

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