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Using data of optic sensors and pigment content in leaves for efficient diagnostics of nitrogen nutrition

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Abstract. Opportune monitoring and diagnostics of a condition of crops permit to make prompt and proper activities on dressing nitrogen fertilizers. This will allow the plants to use the nitrogen applied efficiently, and therefore reduce their use in field. Since nitrogen that has not been utilized by plants is able to escape into the atmosphere or be washed out of the soil with water. The most accurate diagnostic method is to determine the chemical composition of plants, but it takes quite a long time and requires laboratory conditions, which is not always possible in the field. One of the promising methods is photometric diagnostics of crops using optical instruments. Experiment is carried out in contrasting weather conditions, on soddy-podzolic soil with spring barley and spring rapeseed being investigated. Results of research show the efficiency of using optic sensors (N-testers) for efficient diagnostics of nitrogen nutrition of plants. The readings of the device (N-tester) were compared with the concentration of *a* and *b* chlorophyll, determined by a chemical method. Results of diagnostics with portable photometric device 'Yara' are correlating with concentrations of chlorophylls *a* ($r = 0.96$) and *b* ($r = 0.91$) in spring rapeseed. Moreover, correlation of rapeseed yield and concentrations of chlorophylls *a* and *b* has quantity and inverse relation similar to device indication ($r = -0.81$ and $r = -0.70$ respectively). Results of diagnostics with N-tester 'Spectroluxe' are strongly correlating with chlorophyll concentration. Device indication correlates stronger with chlorophyll *b* concentration in spring barley and chlorophyll *a* concentration in spring rapeseed (rapeseed was investigated in dryer conditions). Thus, such a modern optical device as N-tester, whose action is based on measuring the concentration of leafy chlorophyll, can replace chemical methods and increase the efficiency of nitrogen fertilization, which means increasing the productivity of plants and reducing the negative impact of unreasonable use of nitrogen fertilizers.

Key words: chlorophyll fluorescence, fertilizers, optical sensors, nitrogen status, ammonium nitrate, spring barley, spring rape, plant diagnostics.

INTRODUCTION

An increasing the crop yields and the efficiency of fertilizers applied were the main goals of scientists at all times. Often, high doses of nitrogen fertilizers are used in tillages that are an insurance against ignorance of the soil fertility degree. This practice leads to a decrease in the efficiency of nitrogen utilization by plants, an excess nitrogen residue in soil and in crop products (Ferguson et al., 2002; Hashimoto et al., 2007; Tremblay, 2012; Hatamian, 2020). Determination of the nitrogen concentration in soil does not always give the desired result, since nitrogen is quite labile in soil (Gamzikov, 2018). At the same time, plants are a fairly accurate indicator in determining their availability of nitrogen. Various types of checking are used to diagnose plants and quickly make decisions regarding dressing of nitrogen fertilizers. The simplest is visual diagnostics. When an agronomist assesses the condition of crops according to his practical experience, which is not always correct. Chemical diagnostics of plants began to be carried out using a field portable laboratory. Such a diagnostic involves the analysis of fresh plant samples without ashing to determine the content of inorganic forms of elements in them (Shchuklina et al., 2021). To obtain the result, one drop of a 1% solution of diphenylamine dissolved in H_2SO_4 is applied to a cut of stem taken from the upper tier of plant. The results are evaluated in points: 1 point - the drop is colorless or pale blue (severe lack of nitrogen); 2 points - the drop turns blue (the average need of plants in nitrogen); 3 points - the drop is evenly colored in a thick blue-violet color (plants have little need for nitrogen or sufficient nitrogen supply). This method accurately determines the condition of plants, but requires skills in working with acids and is limited both by weather conditions and the agronomist's time. Since these two methods have their drawbacks, another method was developed for determining the supply of plants with nitrogen, using photometric instruments. The method of estimating of nitrogen level in plants with the help of photometric devices is based on measuring of chlorophyll concentration. Concentration measurement is based on intensity of chlorophyll fluorescence and on transparency of leaf plate (Avagyan, 2010).

Monitoring of nitrogen utilization in plants using of optic devices is performed in many countries (Lunagaria et al., 2015, Shchuklina et al., 2021). The process of biosynthesis of organic matter in plant cells and tissues depends on the chlorophyll content, a pigment that determines the functioning of photosynthesis. Both of these factors depend on the amount of chlorophyll in leaf cells. Currently, there are five chlorophyll forms discovered: *a*, *b*, *c*, *d*, *f* (Ke et al., 2021). These forms are found in plants, algae and cyanobacteria. Chlorophyll *a* are detected in plants, chlorophyll *c* is found only in some algae, and chlorophylls *d* and *f* are only in some cyanobacteria (Li & Chen 2015). Chlorophyll is cholorophyllinic acid ester with methyl (for chlorophyll a) or aldehyde (for chlorophyll b) group. Chlorophylls *a* ($C_{55}H_{72}O_5N_4Mg$) and *b* ($C_{55}H_{70}O_6N_4Mg$) both contain four nitrogen atoms, so chlorophyll concentration in plants depends on the amount of nitrogen in plants (Eroshenko et al., 2019). Moreover, concentration of chlorophyll in cells is related to water conditions and other abiotic factors (Afanasiev et al., 2013).

According to the implementators, recently developed commercial optical devices are able to determine the chlorophyll content in leaves regardless of weather conditions, soil pollution levels, and biomass conditions (Samborski et al., 2009; Tremblay, 2010). However, usually the norms of applying of nitrogen fertilizers for a particular crop are advisory in nature. Rationale of the application of optical instruments in agriculture will greatly facilitate the demand of the agronomist in monitoring the agricultural plant condition and diagnosing deviations. This will lead to a more rational use of nitrogen fertilizers, reducing the risk of environmental pollution with residual nitrogen and the unwanted accumulation of pesticides in crop products.

The objective of research was to justify the usage of photometric devices for the analysis of nitrogen level in plants and to investigate the relation of pigment content in leaves to nitrogen nutrition of plants.

MATERIALS AND METHODS

The experiment was carried out in Field experimental station of Russian State Agrarian University - Moscow Timiryazev Agricultural Academy (Moscow, Russian Federation). Soil was loam, soddy-podzolic (Table 1). Soil had high content of available phosphorus (P_2O_5), average content of soluble potassium (K_2O) and low content of humus (1.9%).

Table 1. Soil agrochemical characteristics (0–0.2 m)

Year	pH_{KCL}	Humus, %	N_{EH}	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	P_2O_5	K_2O
			mg kg^{-1} of soil				
2008[1]	4.8	1.9	-	-	-	283	134
2011[1]	5.5	1.7	67.2	4.2	8.7	304	83

[1] Data from Afanasiev R.A. (Afanasiev, 2008; Afanasiev, 2013).

Spring rapeseed (*Brassica napus* L.) cultivar 'Vikros' (2010) and spring barley (*Hordeum vulgare* L.) cultivar 'Mikhailovsky' (2012) were used in the study. The scheme of experiment included spring soil dressing with increasing doses of NH_4NO_3 from 30 kg of active component per hectare to 150 kg of active component per hectare. There were four replications in test and randomized arrangement of variants. Control groups for every cultivar were variants without soil dressing. Crop management practice, accepted for Central region of Non-chernozem zone (Russian Federation). Optic instrument for measurement of chlorophyll concentration in leaves 'Yara' (Konica Minolta, Japan) and experimental optic N-tester 'Spectroluxe' (SPA 'Spectroluxe', Russia) were used. To determine the amount of chlorophyll on the day of measurement with optical sensors, 10 flag leaves from individual plants were selected diagonally from each experimental plot. One medium sample was taken from the crushed mass of one flag leaf. The amount of chlorophyll in leaves was estimated by method, developed by Department of Plant Physiology of Russian State Agrarian University (Tretyakov et al., 1990). Pigment extraction from plants involved the usage of ethanol. Chlorophyll concentration was estimated by spectrophotometer 'Helios Omega' (Termo Scientific Spectronic, the USA) in the laboratory of Pryanishnikov institute of agrochemistry.

Agrometeorological conditions during vegetative period in 2010 were one of the driest for the last 70 years in Central region of Russian Federation. Air drought lasted longer than 50 days (middle June - middle August). During this period precipitation was about 51.6 mm (Fig. 1). According to average annual recordings, 158 mm of precipitation falls during the same period of time (Belolyubtsev & Sukhoveeva, 2012). Drought was accompanied by the temperatures higher than the average annual recordings say. In the third decade of July the temperature was 10.4 °C higher than the average value, and in the third decade of August the temperature was 11.7 °C higher than the average temperature. All these weather conditions result in decrease in spring rapeseed yield.

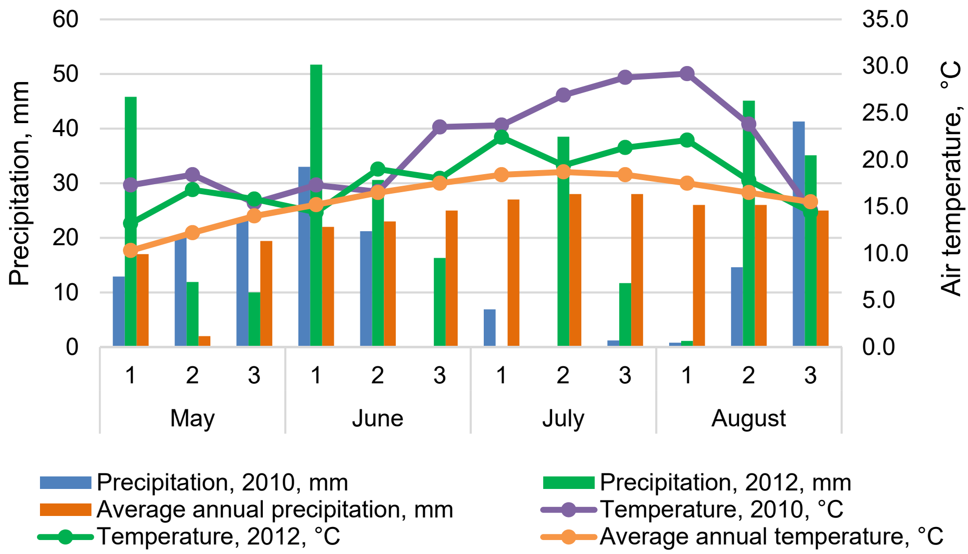

Figure 1. Meteorological conditions of spring rape and spring barley (2010, 2012).

Meteorological conditions in 2012 were favorable for growth and development of spring barley. However, there were periods, lacking in precipitation (first decades of July and August), and periods with the amount of precipitation almost two times higher than the average annual value (first decade of June, second decade of August). Precipitation that fell during the milky stage of spring barley (code BBCH 73-75) led to partial lodging of cereals, which influenced the quality of harvest and the crop yield.

RESULTS AND DISCUSSION

Agrometeorological conditions such as amount of precipitation and air temperature, optimal for growth and development, highly influence crop yield (Cosentino et al., 2012). Other factors such as cultivar and dressing have strong influence on crop yield as well (Turko et al., 2018). In 2010, conditions were abnormal for rapeseed growth not only because higher air temperature, which exceeded the average annual value by 3–12 °C, but because of lack of precipitation as well. Lack of humidity is the main factor that prevents the realization of plants genetic potential (Boyer, 1982). Moreover, the drought stress leads to reducing in assimilation and changes in the process of respiration (Yin et al., 2006). Drought or water stress can significantly reduce plant growth the yield (Shooshtari et al., 2020). At the same time, optimal water conditions are detrimental for efficient usage of nitrogen fertilizers (Belousova et al., 2015).

Weather conditions led to noticeable decrease in rapeseed yield in general. The crop yield equaled to 0.96–1.40 kg ha^{-1} (Fig. 2). Yield of rapeseed, grown without nitrogen soil dressing, was 0.28–0.44 kg ha^{-1} lower than yield of rapeseed, grown with nitrogen fertilizers.

Estimation of chlorophyll concentration in spring rapeseed leaves was carried out during flowering stage (BBCH 57). There was no precipitation three weeks before the estimation. There was 0 mm of precipitation in the third decade of June and 6.9 mm in the first decade of July, which is three times lower the average annual value. However, there was enough soil moisture for normal vital functions of plants and uptake of nitrogen fertilizers. The chlorophyll concentration sequentially increased as the dose of nitrogen fertilizers increased (Fig. 1). Correlation coefficient for chlorophyll *a* equalled 0.94. The increase in chlorophyll *b* concentration occurred in steps. In control group and the group, which obtained the lowest dose of nitrogen (N_{30}), chlorophyll *b* concentration was 0.32–0.37 mg per g, and chlorophyll *b* concentration was 0.49–0.52 under the dose of nitrogen N_{60}. Correlation coefficient for chlorophyll *b* was 0.71.

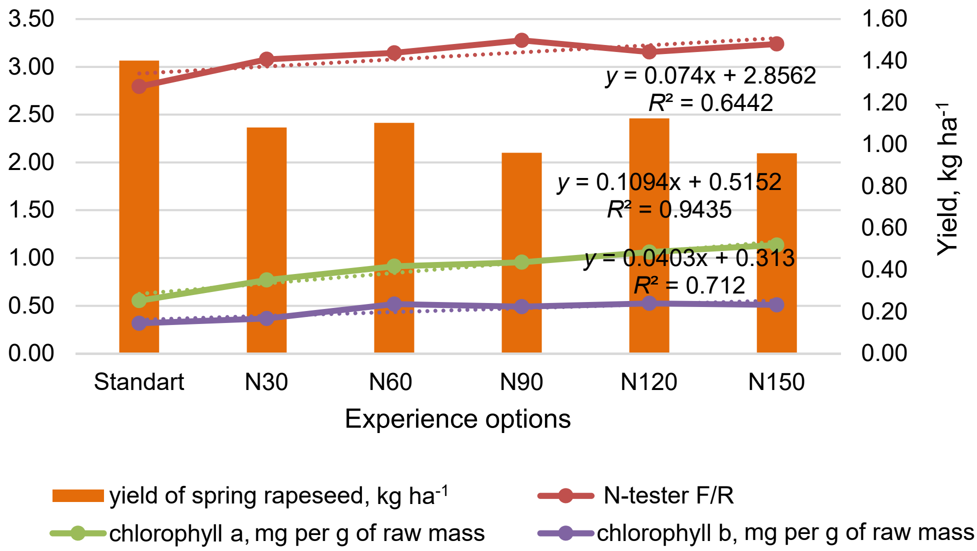

Figure 2. The dependence of the chlorophyll *a* and *b* content and the readings of the Spectrolux N-tester on nitrogen doses on spring rapeseed (2010).

The nitrogen level was measured as well. Measurement was carried out with N-testers 'Spectroluxe' and 'Yara'. The working principle of these instruments is based on the measurement of chlorophyll fluorescence, which represents the amount of chlorophyll in leaves indirectly (Goltsev et al., 2014). The usage of fluorometric methods allows evaluate plants reactions under different stresses: high and low temperatures, drought, saltiness etc. (Fracheboud & Leipner, 2003; Kalaji & Pietkiewicz, 2004; Massacci, 2008). According to results, collected with 'Spectroluxe' in the beginning of July, chlorophyll fluorescence sequentially increased from 2.79 (control group) to 3.28 (N_{120}) with the increase of dose of nitrogen fertilizers. There is a small decrease in fluorescence in groups that consumed higher amounts of nitrogen. N-tester 'Spectroluxe' data strongly correlated with chlorophylls *a* and *b* concentration values (0.89 and 0.84 respectively, Table 2). Moreover, correlation of 'Spectroluxe' data and crop yield was even stronger, but inverse (-0.97), since the rapeseed yield decreased as the dose of nitrogen fertilizers increased. Data from all of the devices used strongly correlated with chlorophyll concentrations. The strongest correlation between chlorophyll *a* concentration and N-tester 'Yara' was shown. The chlorophyll *a* concentration correlated with testers' data as strongly as the sum of *a* and *b* chlorophyll concentrations does. This is because chlorophyll *a* is more present in chloroplasts and has stronger fluorescence, which can be registered by fluorometers (N-testers) (Goltsev et al., 2014).

Table 2. Correlation of N-testers' data and crop yield with the chlorophyll content in spring rapeseed leaves (*: $p < 0.05$)

Parameter	Chlorophyll *a*	Chlorophyll *b*	Sum of the chlorophylls *a* and *b*
Crop yield	-0.81	-0.70	-0.79
N-tester 'Spectroluxe'	0.89	0.84	0.89
N-tester 'Yara'	0.96	0.91	0.96

The water status of spring barley was much better than the water status of spring rapeseed, which was favorable for the crop yield of the barley. However, abundant precipitation, which fell in the beginning of maturing, led to the lodging of the cereals in the groups that received nitrogen doses higher than 90 kg per hectare, which is characteristic for barley (Chen et al., 2014). The lodging of the cereals in some experimental plots was as high as 75%. As a result, the highest crop yield (4.41 kg ha^{-1}) was in experimental plots, where the nitrogen concentration in fertilizer was 60 kg per hectare (Fig. 3), since the lodging of the cereals was not as severe.

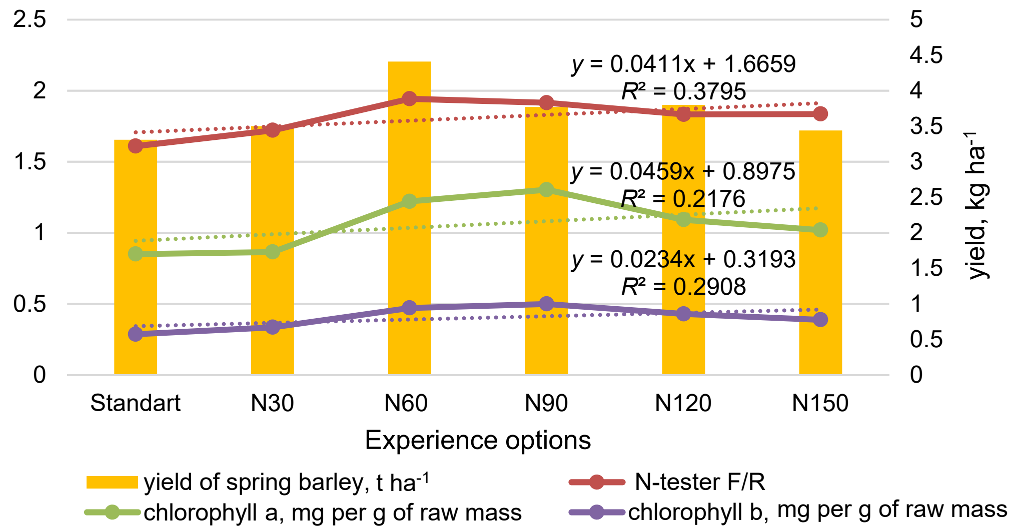

Figure 3. Dependence of chlorophyll a and b content and the readings of the Spectrolux N-tester on nitrogen doses on spring barley (2012).

The measurement of chlorophyll fluorescence and pigments content in spring barley appeared to be useful in the estimation of drought tolerance of new cultivars (Li, 2006). Results, obtained with N-tester 'Spectroluxe' in earing phase (code BBCH 51-52) correlated highly with the chlorophyll concentration in barley leaves. But this correlation was higher in the case of chlorophyll *b*, compared to chlorophyll *a* (Table 3). Data, received from N-tester 'Yara', poorly correlated with chlorophyll concentration. This correlation was higher in the case of chlorophyll *b* ($r = 0.29$). Despite the influence of the lodging of the cereals on the crop yield, strong correlation of the crop yield of barley with the amount of chlorophyll was noted. In the case of chlorophyll *a*, the correlation coefficient equaled 0.73, and for the chlorophyll *b* the correlation coefficient was 0.75.

Table 3. Correlation of N-testers data and crop yield with the chlorophyll content in spring barley leaves (*: $p < 0.05$)

Parameter	Chlorophyll *a*	Chlorophyll *b*	The sum of chlorophylls *a* and *b*
Crop yield	0.73	0.75	0.74
N-tester 'Spectroluxe'	0.92	0.96	0.94
N-tester 'Yara'	0.16	0.29	0.20

The number of green pigments in barley leaves weakly correlated with increasing doses of nitrogen. However, high correlation between chlorophyll *b* concentration and increasing doses of nitrogen was noted ($r = 0.29$).

CONCLUSIONS

Chemical diagnostics (the concentrations of chlorophyll and nitrogen in leaves) is the most precise method of estimation of nitrogen status of plants. However, these methods require a lot of time, laboratory conditions, availability of special devices and reagents, including acids, and skills of working with these reagents (Hatamian et al., 2018; Souri et al., 2018). Modern optical instruments, which working principle is based on chlorophyll concentration measurement, can substitute chemical methods. In the present study results of diagnostics with portable photometric instrument 'Yara' strongly correlate with the results of measurement of green pigments concentration in rapeseed leaves by chemical methods. The correlation coefficient for chlorophyll *a* and the sum of chlorophylls *a* and *b* reached -0.96, and the correlation coefficient for chlorophyll *b* was -0.91. Moreover, the data from 'Yara' weakly correlated with the number of pigments in barley leaves. Results of diagnostics, received due to the new device 'Spectroluxe', highly correlate with pigment content in rapeseed and barley leaves. However, it is necessary to conduct research, evaluating the cultivars in certain conditions, to determine the dose of nitrogen fertilizers applied.

Our study confirms the results obtained earlier by other researches and will contribute to the development of algorithms for converting the readings of optical sensors into real recommendations for the application of nitrogen fertilizers to various crops grown in different soils and climatic conditions.

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