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Effects of nitrogen fertilizers on wheat photosynthetic pigment and carbohydrate contents

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The effect of nitrogen fertilizers on the physiological indices of winter wheat (*Triticum aestivum* L. variety 'Ada') was investigated in experimental fields of the Lithuanian University of Agriculture in 2005–2006. The rates of fertilizers during plant vegetation were as follows: N_{90}, N_{150} and N_{180}. In sowing time, wheat was fertilized with $N_{30}P_{80}K_{120}$. In tillering stage, plants were fed with calcium-ammonium nitrate N_{60} and N_{80}. In booting stage, they were fertilized through leaves with carbamate solution N_{30} and N_{40}. Wheat organogenesis stage, photosynthetic pigment and carbohydrate contents were determined. According to our results, plants respond to nitrogen supply differently during separate phenological phases. Supplementary fertilization accelerated plant development only at lower applied concentrations (N_{150}) and developmental differences showed up only in booting stage. The best development was related with a high chlorophyll a / b ratio and a low carbohydrate content. Total photosynthetic pigment contents increased with plant age and were higher at higher fertilization rates. Chlorophyll a, b and carotenoid biosynthesis showed similar responses to N fertilization. The increase in photosynthetic pigment contents coheres with a decreased fructose accumulation and an enhanced disugar (sucrose and maltose) synthesis intensity during wheat growth. The results confirm the potentiality to control wheat physiological indices by using different nitrogen fertilization designs.

Key words: nitrogen fertilizers, carbohydrates, photosynthetic pigments, winter wheat

INTRODUCTION

Fertilization is the main tool of agricultural engineering. It has a strong effect on productivity, nutritional quality management and the regulation of harvest formation processes [1, 2]. Nitrogen fertilizers are among the most important and effective implements in agriculture, stimulating a lot of vital processes in plants. The amount of nitrogen applied to plants must be carefully managed to ensure that N will be available throughout the growing season and the vegetative and reproductive development will be not restricted [3]. It is of ecological and economic importance to spread higher nitrogen fertilizer doses over few times, and in later developmental periods to fertilize through leaves [4]. Fertilization through leaves intensifies photosynthetic processes; plants assimilate nitrogen faster [1], although nitrogen uptake and utilization are determined by wheat genotypic differences and linked to a variety of morphological and physiological factors, including the length and activity of the root system, the intensity of nitrate uptake, activity of nitrate reductase, sink activity of grains, carbohydrate production and N losses [5].

Plant and crop productivity are determined by closely interrelated growth and development processes. The intensity of photosynthesis and respiration, assimilating area, photosynthetic productivity changes during them [6, 7]. A photosynthetic capacity of leaves is closely related to their nitrogen content, and chlorophyll quantity is a very stabile parameter for soil nitrogen uptake estimation [7]. Carbohydrate metabolism is also determined by the availability of nitrogen as the major limiting factor [8]. Carbohydrates perform important hormone-like functions as primary messengers due to their essential role in plant growth, development and metabolic links with the initial physiological processes [9]. Therefore, the observation of crop photosynthetic indices allows optimizing plant cultivation technological processes [10].

The performance of the photosynthesis system and the effect of nutrients were explored in different ways. At present, there is no general physiological model for selecting the optimal fertilization regime. Therefore, the object of this study was to determine the effect of main and supplementary fertilization on winter wheat development and photosynthetic pigment and carbohydrate contents in plant fresh weight.

MATERIALS AND METHODS

Experiments were performed at the experimental station of the Lithuanian University of Agriculture in 2005–2006. The soil

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Table 1. **Meteorological conditions during field experiments. Records of Kaunas Meteorological Station, 2005–2006**

	2005 / 2006											
Months	09	10	11	12	01	02	03	04	05	06	07	08
Indexes	Temperature °C											
Average	14.2	8.0	2.8	–1.6	–7.2	–6.3	–2.7	6.5	12.5	16.5	20.9	17.8
Perennial	12.2	6.8	1.5	–3.3	–5.0	–4.3	–0.8	6.0	12.3	15.5	17.5	16.4
Indexes	Precipitation (mm)											
Total	46.5	10.8	25.0	46.1	19.7	17.7	21.9	29.3	74.5	18.0	70.7	165.6
Perennial	52.4	50.0	45.7	35.9	30.6	27.8	32.4	38.5	53.4	62.8	81.6	70.0

Table 2. **Experimental design**

Treatment	Fertilization time			
	Sowing	Tillering stage BBCH 23–25	Booting stage BBCH 34–36	Milky ripeness stage BBCH 71-74
N_{90}	N_{30}	N_{60}		
N_{150}	N_{30}	N_{60}	N_{30}	N_{30}
N_{180}	N_{30}	N_{80}	N_{40}	N_{30}

of the plot was Calcari-Epihypogleyic Luvisol (LVg-p-w-cc). According to agrotechnical characteristics, the arable layer of soil before experiment installation was neutral, of medium humus, high phosphorus and potassium contents.

The years 2005/2006 were not favorable for wheat growth (Table 1). There was enough heat and humidity in the time of sowing, although in October and November there were less precipitation than the perennial average. Average temperatures in January, February and March were lower than the perennial average, although wheat did not suffer. In July, in the last plant developmental stages, in all territory of Lithuania there was a drought, and wheat sallowed earlier.

The winter wheat (*Triticum aestivum* L.) variety 'Ada' was investigated. The seed rate was 5 mill. viable seeds per ha. The experiment was performed in four replications arranged in a randomized order. The total plot area was 36 m^2 the trial plot being 26.4 m^2. Soil was prepared according to winter wheat agrotechnical requirements. The experimental design is presented in Table 2. In the sowing time, winter wheat was fertilized with complex fertilizers $N_{30}P_{80}K_{120}$. During vegetation, nitrogen fertilization rates were as follows: N_{90}, N_{150} ir N_{180}. In the tillering stage, plants were fertilized with calcium ammonium nitrate N_{30} and N_{40}. In the milky ripeness stage, plants were fertilized through leaves with carbamide solution N_{30}.

Photosynthetic pigment (chlorophyll a, b and carotenoids) content in fresh matter (FM) was determined in 100% acetone extract by the spectrophotometric Wettstein [11] method using a Genesys 6 spectrophotometer (ThermoSpectronic, USA). Samples for saccharide analysis by the high performance chromatographic method were prepared grinding about 1–2 g of fresh plant weight and diluting with 4 ml of hot bidistiled water. Extraction proceeded for 12 h, and then a sample was filtered through 0.2 μm acetate cellulose filters. Fructose, glucose, sucrose and maltose analysis was performed using the Shimadzu HPLC 10A chromatographic system (Shimadzu, Japan) with a refractive index detector, Adsorbosil NH_2 column (150 × 4.6 mm) (Alltech, USA). Mobile phase: 75% acetonitrile.

The organogenetic stages were determined according to Kuperman's [12] developmental periodization and methodology.

Data quantification and statistical analysis were performed using MS Excel software. Data error bars presented in Fig. 1 are a standard deviation of three biological sample measurements and in Fig. 2 a standard deviation of five analytical measurements.

RESULTS

In spring, in the tillering stage when plants were fertilized only in at sowing, 80% of wheat apical meristems were in organogenesis stage IV and 20% in organogenesis stage III. After supplementary fertilization at the tillering stage with different nitrogen fertilizer rates, all plants developed equally in the beginning of the booting stage (organogenesis stage V^b). When wheat was not fertilized in the booting stage, it also developed equally (organogenesis stage V^c), and when plants were additionally fertilized with nitrogen a better development was observed in the N_{30} + N_{60} + N_{30} treatment: 60% of wheat reached organogenesis stage VI when in the N_{30} + N_{80} + N_{40} treatment most of the plants were in V^b organogenesis stage (Table 3).

Analysis of photosynthetic pigments showed (Fig. 1 A–C), that the total contents of chlorophyll a, b and carotenoids increased with plant age, though the chlorophyll a/b ratio decreased. However, supplementary fertilization positively affected the chlorophyll ratio, and the decrease was smaller as compared to reference. In the booting stage, the differences in photosynthetic pigment contents were statistically insignificant between N_{30} + N_{60} and N_{30} + N_{80} fertilization treatments, although in the latter developmental stages analysis showed distinct differences. In the N_{30} + N_{60} treatment when plants were fertilized only in the tillering stage, no significant changes were observed in chlorophyll and carotenoid contents in the flowering stage, although

Table 3. **Organogenesis stages of winter wheat 'Ada' under different fertilization regimes**

Fertilization combinations	Fertilization time		
	Tillering stage	Beginning of booting	Booting stage
N_{30}	III – 20%; IV – 80%		
$N_{30} + N_{60}$		V^b – 100%	V^c – 100%
$N_{30} + N_{80}$		V^b – 100%	
$N_{30} + N_{60} + N_{30}$			V^c – 40%; VI – 60%
$N_{30} + N_{80} + N_{40}$			V^b – 80%; V^c – 20%

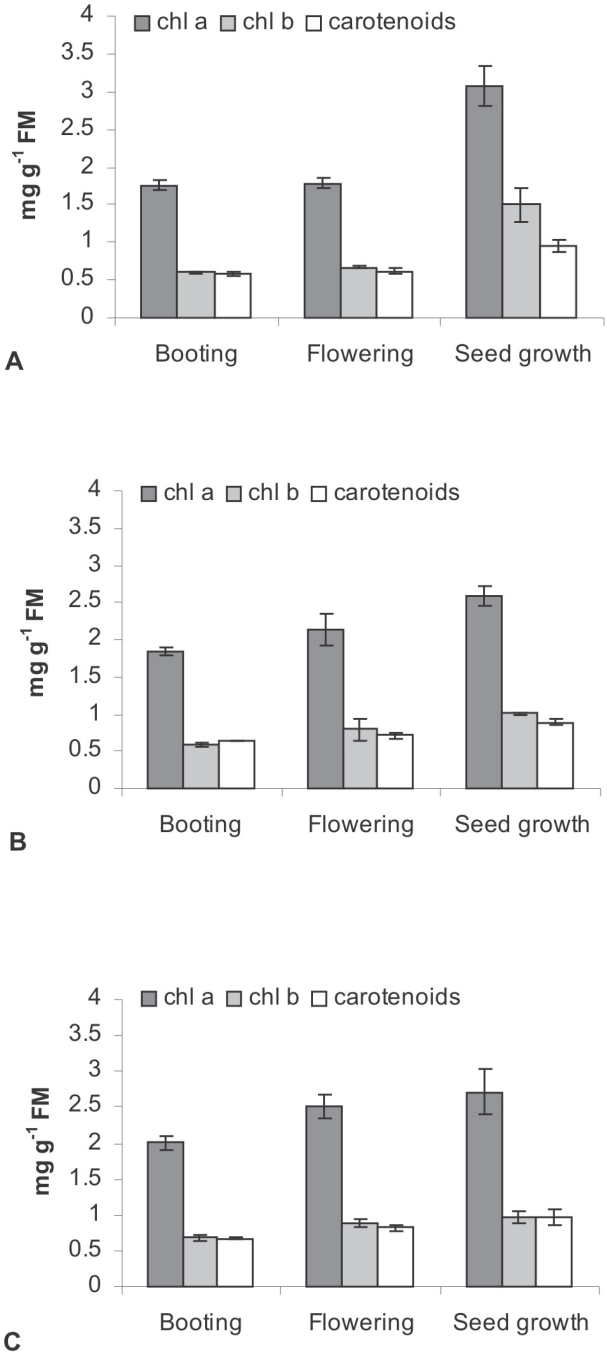

Fig. 1. Effects of nitrogen fertilizers on wheat photosynthetic pigment contents. A – plants fertilized with $N_{30} + N_{60}$, B – plants fertilized with $N_{30} + N_{60} + N_{30}$, C – plants fertilized with $N_{30} + N_{80} + N_{40}$

in the seed growth stage the content of chlorophyll a and b increased by the factor of 1.75 and 1.51, respectively, as compared to the booting stage. Supplemental fertilization through leaves with carbamide (Fig. 1 B, C) stimulated the accumulation of chlorophylls and carotenoids in leaves, and the higher nitrogen rate (N_{40}) evoked a more intensive synthesis of photosynthetic pigments in the flowering stage, although, in the seed growth stage, after supplementary fertilization ($N_{30} + N_{60} + N_{30}$ and $N_{30} + N_{80} + N_{40}$), the concentrations of chlorophyll a and b were significantly lower than in the reference treatment ($N_{30} + N_{60}$).

Quantitative and qualitative changes in leaf carbohydrate contents were observed during wheat development (Fig. 2). In the booting stage, irrespective of fertilization treatment, fructose was the dominant sugar, although in the later developmental phases fructose contents decreased and disugar (sucrose and maltose) contents rose. Supplementary nitrogen fertilization affected leaf carbohydrate contents unequally in different phenological phases. In the booting stage, more fructose and total carbohydrates were accumulated in $N_{30} + N_{60}$ treatment. Supplementary fertilization inhibited carbohydrate synthesis: there were about 2.2 times less fructose and maltose and no sucrose in $N_{30} + N_{60} + N_{30}$ treatment as compared to reference.

When the plants were flowering, sugar contents decreased almost three times in the reference treatment ($N_{30} + N_{60}$), although in the seed growth, fructose contents decreased and disugar contents increased (Fig. 2 A). Such trends were observed also in the $N_{30} + N_{60} + N_{30}$ treatment (Fig. 2 B), although the decrease in carbohydrate contents was smaller, and in the seed growth stage glucose contents rose from 0.6 to 3.4 mg g^{-1} as compared to reference. In the $N_{30} + N_{80} + N_{40}$ treatment (Fig. 2 C), higher nitrogen rates, contrarily to previous experiments, stimulated glucose synthesis in the flowering stage, and the total carbohydrate contents were 1.6 times higher as compared to lower rates of supplementary fertilization. In the seed growth stage, sugar accumulation in wheat leaves was significantly inhibited, and the contents were lower than in reference treatment.

DISCUSSION

Nitrogen availability and internal distribution play a critical role in the regulation of various growth-related and morphogenetic aspects of plant development [8]. Nitrate reductase, the major enzyme in nitrogen metabolism, is considered to be a limiting factor for higher plant growth, development and protein production [13]. Plant reaction to N supply is different during separate phenological phases. According to our results, developmental differences were significant only in the booting stage. After

supplementary $N_{30} + N_{60} + N_{30}$ fertilization plants reached the highest organogenesis stage VI and after fertilization with $N_{30} + N_{80} + N_{40}$ the lower organogenesis stage than in the reference treatment. These processes require much photosynthetic energy [14], therefore, balanced nitrogen nutrition is a limiting factor for wheat development.

One of the important factors indicating the efficiency of nitrogen fertilization is the performance of the photosynthetic apparatus that determines photosynthetic pigment contents in leaves [15]. Chlorophylls are very sensitive to changes in nitrogen contents. According to our results, total contents of photosynthetic pigments increased with plant age and were higher at higher fertilization rates. Chlorophyll a, b and carotenoid biosynthesis showed similar responses to N fertilization. However, the chlorophyll a/b ratio decreased in the later developmental stages. Nitrogen showed the most pronounced effect on photosynthetic pigment synthesis in the flowering stage. The photosynthetic capacity of leaves is related to the nitrogen content primarily because of proteins of the Calvin cycle, and thylacoids represent the majority of leaf nitrogen [16].

There were also only a few cases of correlation between sugar and photosynthetic pigment contents. In the treatment where the development was the best ($N_{30} + N_{60} + N_{30}$ in the booting stage) the chlorophyll a/b ratio was the highest and the total carbohydrate content was the lowest. Higher amounts of hexoses preceded the loss of photosynthetic activity and sucrose and starch accumulation. The possibility that sugar repression of photosynthesis under physiological conditions depends more crucially on the C : N status of leaves than on the carbohydrates status alone [17] should be also evaluated.

Increased photosynthesis and assimilate availability emphasize the possible role of carbohydrates in the transition to flowering. In young plants, fructose dominated and reacted to nitrogen sensitively. During booting, monosugar accumulation decreased and sucrose contents significantly increased. Wheat then reached organogenesis stage V^c–VI, and meiosis started. These results support the hypothesis that sucrose is the major sugar acting in generative development processes [18, 19].

Fertilization with nitrogen enables to manage physiological indices during wheat vegetation. Nitrogen metabolism affects wheat photosynthesis and controls plant development, possibly through carbohydrate metabolism which is involved in other important plant signaling and regulatory pathways [8].

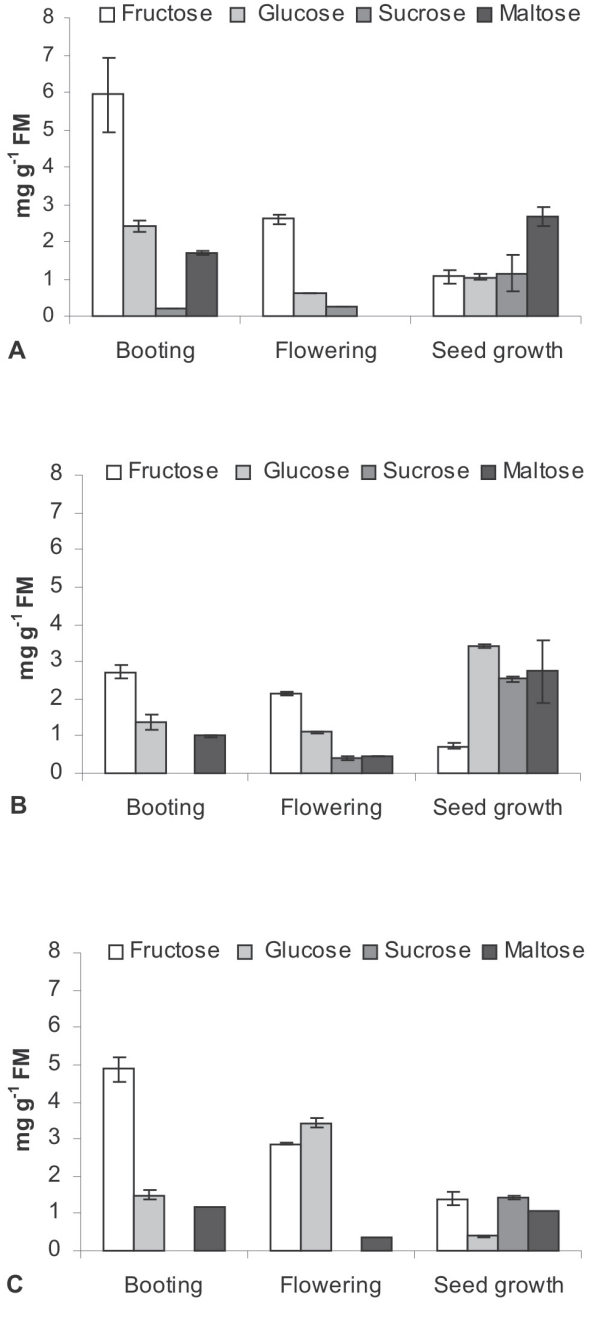

Fig. 2. Effects of nitrogen fertilizers on leaf carbohydrate contents. A – plants fertilized with $N_{30} + N_{60}$, B – plants fertilized with $N_{30} + N_{60} + N_{30}$, C – plants fertilized with $N_{30} + N_{80} + N_{40}$

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Tatjana Tranavičienė, Jūratė B. Šikšnianienė, Akvilė Urbonavičiūtė, Ilona Vagusevičienė, Giedrė Samuolienė, Pavelas Duchovskis, Algirdas Sliesaravičius

AZOTO TRĄŠŲ POVEIKIS ŽIEMINIŲ KVIEČIŲ FOTOSINTEZĖS PIGMENTAMS IR CUKRUI

S a n t r a u k a

Azoto trąšų poveikis žieminių kviečių (*Triticum aestivum* L. vaislė. ‘Ada’) fotosintezės pigmentams ir cukrui tirtas Lietuvos žemės ūkio universiteto bandymų stotyje 2005–2006 metais. Augalų vegetacijos metu naudotos tokios trąšų normos: N_{90}, N_{150} ir N_{180}. Per sėją žieminiai kviečiai buvo patręšti kompleksinėmis trąšomis $N_{30}P_{80}K_{120}$, krūmijimosi metu – kalcio amonio salietra N_{60} ir N_{80}, bamblėjimo tarpsnyje – per lapus karbamido tirpalu N_{30} ir N_{40}. Nustatyti kviečių organogenezės etapai, fotosintezės pigmentų ir cukraus kiekis lapuose. Rezultatai rodo, kad augalo reakcija į azoto trąšas atskirų fenologinių tarpsnių metu yra savita. Papildomas tręšimas paspartino augalų vystymąsi tik veikiant mažesnėmis tirtomis trąšų normomis (N_{150}), o vystymosi skirtumai išryškėjo tik bamblėjimo tarpsnyje. Geriausiai besivystančių augalų lapuose buvo didžiausias chlorofilo a / b santykis ir mažesnis bendras tirtų angliavandenių kiekis. Bendras fotosintezės pigmentų kiekis didėjo augalams augant ir didinant trąšų normas. Fotosintezės pigmentų kiekio padidėjimas siejamas su sumažėjusiu fruktozės kaupimu ir intensyvesne sacharozės ir maltozės sinteze augant kviečiams. Gauti rezultatai patvirtina hipotezę, kad sacharozė yra pagrindinis angliavandenis, jautriai reaguojantis į azoto kiekį augaluose, dalyvaujantis generatyviniame vystymesi ir indukuojantis pokyčius fotosintezės sistemos veikloje. Šių mechanizmų įsisavinimas leistų kontroliuoti kviečių fiziologinius parametrus naudojant skirtingas tręšimo azotu schemas.

Raktažodžiai: azoto trąšos, cukrus, fotosintezės pigmentai, žieminiai kviečiai