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The effect of differential nitrogen fertilization on photosynthetic pigment and carbohydrate contents in the two winter wheat varieties

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Abstract. The effect of nitrogen fertilizers on photosynthetic indices in two winter wheat (*Triticum aestivum* L.) varieties 'Ada' and 'Seda' was investigated in the experimental station of Lithuanian University of Agriculture during 2005–2006 and 2006–2007. The rates of fertilizers during wheat vegetation were as follows: N_{90}, N_{120} and N_{150}. In sowing time both varieties were fertilized with $N_{30}P_{80}K_{120}$. In tillering stage plants were fed with calcium-ammonium nitrate N_{60} and N_{80}. In stem elongation stage they were fertilized through leaves with carbamide solution N_{30} and N_{40}. Wheat photosynthetic pigment and carbohydrate contents were determined in flowering and seed growth stages. The results of analysis show that photosynthetic pigment and carbohydrate status are suitable indicators of the activity of the winter wheat photosynthetic system. Suitably selected nitrogen fertilization design may delay the natural senescence processes. However, the emergence of the indices of senescence is also dependent on environmental conditions. Nitrogen remobilization and photosynthetic activity during natural senescence is cultivar specific. The photosynthetic system of 'Ada' wheat variety is more sensitive to the evocative factors than 'Seda' wheat. The soluble sugars also participate in senescence launching: the lower hexoses/sucrose ratio corresponds to decline in photosynthetic pigment degradation. Earlier photosynthetic senescence corresponds to reduced wheat grain yields.

Key words: winter wheat, photosynthetic pigments, carbohydrates, yield, nitrogen fertilization

INTRODUCTION

Nitrogen is an element limiting plant growth in many ecosystems; efficient use of nitrogen is believed to contribute to the fitness of the plant. Moreover, the nitrogen availability and internal distribution plays a critical role in the regulation of various growth-related and morphogenetic aspects of plant development that are usually attributed to hormonal factors (McIntyre, 2001; Hikosaka, 2004). The amount of nitrogen applied to plants must be carefully managed to ensure that N will be available throughout the growing season, and vegetative and reproductive development will not be restricted (Vidal et al., 1999). It is of ecological and economical importance to spread higher nitrogen fertilizer doses over few times, and in later developmental periods to fertilize through leaves. Proven methods for making nitrogen fertilizer

recommendations exist for the most arable crops. The ones most recently used are based on the evaluation of performance of the photosynthetic system. A positive correlation between leaf N fertilization and rate and the chlorophyll content is well documented for a number of plant species and has been investigated for rapid N determination for most major crops including corn, rice, wheat (Sabo et al., 2002; Bojovic & Stojanovic, 2005; Fritshi & Ray, 2007; Houles et al., 2007). Regulation of metabolic and developmental processes by sugars also often depends on nitrogen supply, suggesting that the sugar and nitrogen signaling pathways interact (Paul & Driscoll, 1997; Wingler et al., 2004). Therefore, the assay of wheat carbohydrate and photosynthetic pigment contents may serve to optimize wheat fertilization technologies.

Genotypic differences in grain yield formation of wheat are linked to a variety of morphological and physiological factors that affect uptake and utilization of nitrogen (Diekmann & Fishbeck, 2005); consequently, the cultivar dependent fertilization regimes should be explored to gain an optimal effect. Therefore, there presently is no general physiological model for selection of the optimal fertilization regime. The objective of this study was to evaluate the effect of the main and supplementary nitrogen fertilization on the performance of photosynthetic systems of two Lithuanian winter wheat varieties: 'Ada' and 'Seda'.

MATERIALS AND METHODS

Experiments were performed in the experimental station of Lithuanian University of Agriculture during 2005–2006 and 2006–2007. The soil of the plot was the Calcari-Epihypogleyic Luvisol (LVg-p-w-cc). According to agrotechnical characteristics the arable layer of soil before experiment installation was neutral, of medium humous, high phosphorus and potassium contents.

The 2005–2006 year was quite favorable for winter wheat growth (Table 1). Temperatures and humidity were suitable for successful germination, and low latter humidity did not affect its development. The winter was quite cold, temperatures dropped to –28°C, although the snow cover was thick and wheat overwintered well. The spring was cold and vegetation renewed late, however the meteorological conditions during the remaining vegetation period were suitable for wheat growth and development. In the 2006–2007 growing season, in the autumn and spring, the humidity and temperatures were higher than the perennial averages. In 2007 vegetation began early, because in March, the temperature exceeded perennial averages by 4.4 degrees. During the remaining vegetation period, the weather was also warmer than usual. The humidity was sufficient; therefore wheat matured and dried off early.

The winter wheat (*Triticum aestivum* L.) varieties 'Ada' and 'Seda' were investigated. The experiment was performed in four replications arranged in a randomized order. Total plot area – 36 m^2, trial plot – 26.4 m^2. Soil was prepared according to winter wheat agrotechnical requirements. The seed rate – 5 mln. viable seeds per ha. The experiment design is presented in Table 2. In sowing time winter wheat was fertilized with complex fertilizers $N_{30}P_{80}K_{120}$. In tillering stage plants were fertilized with calcium ammonium nitrate N_{60} and N_{80}. In stem elongation stage, wheat was fertilized through leaves with Urea solution N_{30} and N_{40}.

Table 1. Meteorological conditions during field experiments. The records of Kaunas meteorological station, 2005–2007 years.

Months	09	10	11	12	01	02	03	04	05	06	07	08
Temperature °C												
Average 2005/2006	14.3	8.0	2.8	-1.6	-7.2	-6.3	-2.7	6.5	12.5	16.5	20.9	17.8
Average 2006/2007	14.6	9.7	4.4	4.0	1.3	-6.2	5.2	7.0	13.6	17.8	17.1	18.5
Perennial Average	12.2	6.8	1.5	-3.3	-5.0	-4.3	-0.8	6.0	12.3	15.5	17.5	16.4
Precipitation (mm)												
Total *2005/2006*	46.5	10.8	25.0	46.1	19.7	17.7	21.9	29.3	74.5	18.0	70.7	165.6
Total 2006/2007	89.8	47.7	48.3	42.6	90.0	30.7	31.4	22.2	96.5	70.0	148.7	78.6
Perennial Average	52.4	50.0	45.7	35.9	30.6	27.8	32.4	38.5	53.4	62.8	81.6	79.0

Table 2. The design of fertilization experiment.

	Fertilization time			
N treatment	**In sowing time**	**Tillering stage BBCH 23 – 25**	**Stem elongation stage BBCH 34 – 36**	**Total N supply (kg N ha^{-1})**
N_{30}+N_{60}	N_{30}	N_{60}		90
N_{30}+N_{60}+N_{30}	N_{30}	N_{60}	N_{30}	120
N_{30}+N_{80}+N_{40}	N_{30}	N_{80}	N_{40}	150

Analysis of photosynthetic pigment contents were performed in flowering stage (BBCH 65-67) and in the seed growth stage (BBCH 71-75), whereas carbohydrate contents were estimated just in flowering stage.

Photosynthetic pigment (chlorophyll a, b and carotenoids) content in fresh matter (FM) was determined spectrophotometrically in 100% acetone extract (Gavrilenko & Zigalova, 2003). Spectrophotometer – "Genesys 6" (ThermoSpectronic, USA).

Samples for carbohydrate analysis in wheat leaves were prepared grinding about 1–2 g of fresh plant matter and diluting with 4 ml of hot bidistilled water. Extraction proceeded for 12 h, and then samples were filtered using paper and 0.2μm acetate cellulose filters. Fructose, glucose, sucrose and maltose analysis was performed by high performance chromatographic method using Shimadzu HPLC 10A chromatographic system (Shimadzu, Japan) with refractive index detector, Adsorbosil NH_2 column (150 x 4,6 mm) (Alltech, USA). Mobile phase: 75% acetonitrile, flow rate: 1ml min^{-1}.

The grain yield was established in each replication plot and expressed as t per ha. The Fisher's LSD criteria ($P = 0.5$) was used to evaluate the yield differences between fertilization treatments.

Data quantification and statistical analysis were performed using MS Excel software. Data error bars presented in Fig. 1 show the standard deviation of the measurements of three biological samples, and in Fig. 2 – the standard deviation of five analytical measurements.

RESULTS AND DISCUSSION

Chlorophyll content is of particular significance to precision in agriculture as an indicator of photosynthetic activity. Nitrogen concentration in green vegetation is related to chlorophyll content, and therefore indirectly to one of the basic plant physiological processes: photosynthesis (Sabo et al., 2002; Bojovic & Stojanovic, 2005). Appealing to our results of analysis of photosynthetic pigments (Fig. 1), it could be premised that the 2006 year was more favorable for wheat growth than the 2007. The greatest chlorophyll content in plants occurs at the outset of the flowering phase and chlorophyll is believed to take part in the processes of organogenesis (Bojovic & Stojanovic, 2005). Remarkably higher contents of chlorophyll *a* in the flowering stage in 2006 indicate better growth conditions, though, in the summer of 2007 wheat developed more intensively and sallowed earlier than in 2006. Therefore, irrespectively of the fertilization treatment, the lower contents of photosynthetic pigments, and lower value of chlorophyll *a* and *b* ratio was determined.

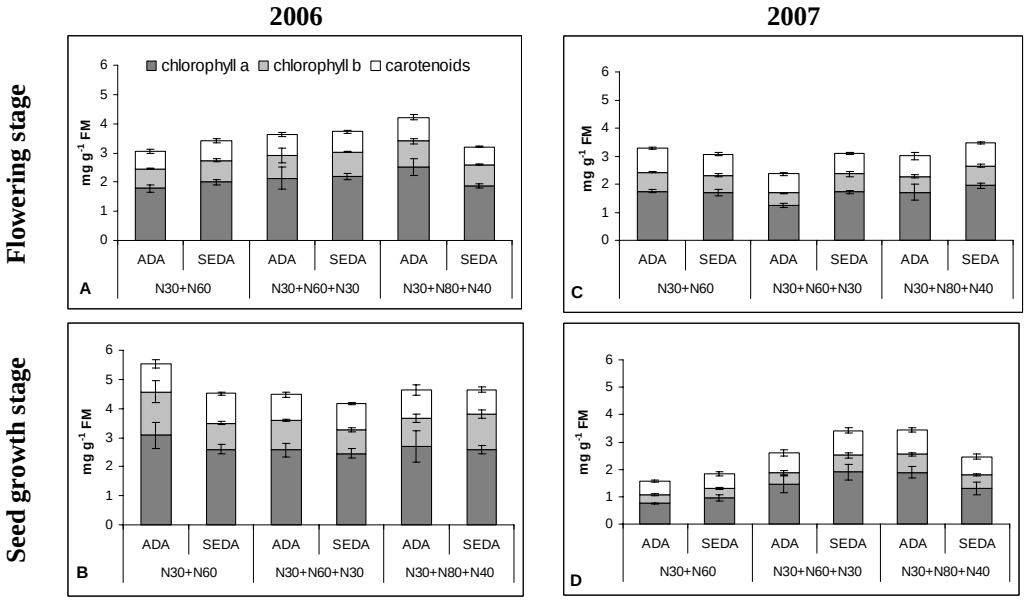

Fig. 1. Photosynthetic pigment contents in the leaves of winter wheat. A,B – results of experiments performed in 2006, C,D – results of experiments performed in the summer of 2007 year.

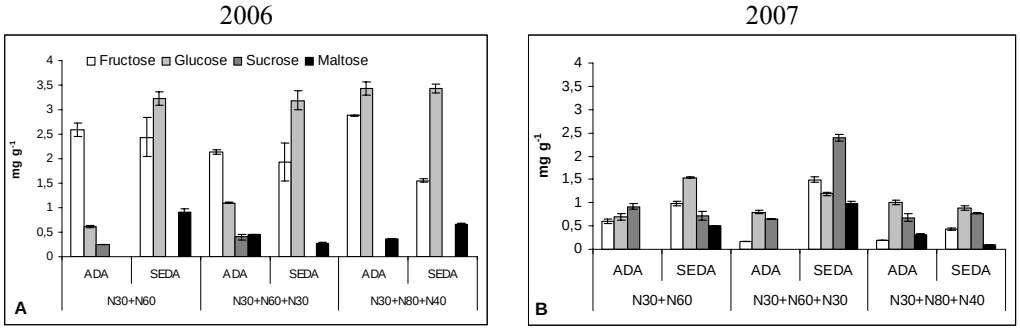

Fig. 2. Carbohydrate contents in the leaves of winter wheat in the flowering stage. A – results of experiments performed in 2006, B – results of experiments performed in the summer of 2007 year.

The chlorophyll content in the seed growth stage was higher as compared to the flowering stage in 2006 and about 2 times lower in 2007. Such a decline in photosynthetic pigment content could indicate the earlier processes of physiological senescence (Horensteiner & Feller, 2002; Wingler et al, 2004; Wingler et al., 2006). Senescence represents the final stage of leaf development and is characterized by the transition form nutrient assimilation to nutrient remobilization. The rate of senescence and the remobilization of leaf nitrogen is related to the nitrogen nutrition status of the plant and on source/sink relations (Hortensteiner & Feller, 2002). The ratio of chlorophyll *a* and *b* quantum and carotenoid contents is a suitable senescence indicator. In 2006, in flowering stage, irrespectively to the nitrogen nutrition it varies between 2.9 and 3.6; in 2007 – from 2.1 to 2.55 mg g^{-1}. In 2007 it also decreases remarkably in seed growth stage, showing diminished activity and efficiency of the photosynthesis system. Supplemental fertilization through leaves in the stem elongation stage competitively breaks the chlorophyll degradation.

No tendentious differences in photosynthetic pigment contents between two investigated varieties were observed. However, 'Ada' wheat pigment accumulation in leaves was more sensitive to different fertilization regimes.

Senescence in winter wheat begins in the end of organogenesis stage VIII. It is stated that senescence is a sugar-regulated process, involving hexokinase as sugar sensor and dependent on plant nitrogen status (Horensteiner & Feller, 2002; Wingler et al., 2004; Gibson, 2005; Wingler et al., 2006). However, the carbohydrate status in the flowering stage in leaves is completely different between investigated years (Fig. 2). In 2006, hexoses (fructose and glucose) are the dominating sugars and their contents vary depending on the nitrogen fertilization regime. In the leaves of 'Ada' wheat, when plants were fertilized with N30+N60 and N30+N60+N30, some sucrose was detected; this sugar did not accumulate in the leaves of 'Seda' wheat.

In 2007, supporting the results of photosynthetic pigment analysis, the decreased total indicated carbohydrate contents and the decreased hexoses/sucrose ratio, representing hexokinase activity, indicates the presumable senescence processes. The lower chlorophyll and carotenoids ratio, together with decreased sugar accumulation in 'Ada' wheat leaves show more intense proceeding of decline in photosynthesis. 'Seda'

was less affected by the nitrogen and by other environmental factors which promoted senescence.

The performance of the photosynthesis system is directly defined by the meteorological conditions of the year. The successful germination and favorable conditions during the early developmental stages in 2005 were a fine outset for further growth and development processes and the low winter temperatures, much precipitation, cold spring did not affect the wheat negatively, as compared to 2006–2007; when redundant temperatures and humidity in the autumn-winter season caused worse germination and the poor start hindered the complete growth cycle potential. Due to the warmer wintering period plants did not reach full rest phase, and higher spring temperatures evoked earlier developmental changes in wheat. However, the humid and warm summer did not equilibrate the previous losses.

The worse meteorological conditions in 2006–2007 year season affected wheat organogenesis and determined the earlier physiological senescence in the wheat photosynthesis system, thus the yield in this year (Table 3) was less by the factor of 1.3–1.6 t ha^{-1}, as compared to the yield of 2005–2006. The yield of 'Ada' wheat was more dependent on the fertilization regime than 'Seda'; and the grain yield loss due to the senescence was larger in the 'Ada' wheat.

Table 3. The effect of nitrogen fertilization on wheat yield, t ha^{-1}.

Treatment	Ada		Seda	
	2006	**2007**	**2006**	**2007**
$N_{30}+N_{60}$	6.92	4.34	8.00	4.81
$N_{30}+N_{60}+N_{30}$	8.40	5.93	9.22	5.91
$N_{30}+N_{80}+N_{40}$	7.93	5.90	9.14	5.69
$LSD_{0.5}$	0.36	0.27	0.42	0.24

Nitrogen is not the sole factor affecting senescence in wheat leaves (Salvagiotti & Miralles, 2007). Environmental, meteorological conditions are factors in the alterations of the performance of natural aging in wheat plants. However, the suitably designed fertilization regime not only affects the balance between growth and development processes, optimal yield formation, but also delays natural senescence in wheat, prolonging the period of intense photosynthesis, completing the source – sink transport, thereby inducing metabolite accumulation and the raise in grain mass.

CONCLUSIONS

1. Photosynthetic pigment contents and carbohydrate status of winter wheat leaves are proved to be suitable indicators of the performance of photosynthetic system and probable senescence.
2. Nitrogen nutrition is not the only factor affecting natural physiological leaf senescence. Our results show that the changeable environmental conditions in different years can suspend or accelerate the decline in photosynthesis and reduce the grain yield. Suitably assorted nitrogen fertilization regimes may delay these processes.
3. Nitrogen remobilization, photosynthetic activity and grain yield formation during natural senescence is cultivar specific. The photosynthetic system of

'Ada' wheat variety is more sensitive to the evocative factors than 'Seda' wheat.

4. The soluble sugars also participate in senescence launching: the lower hexoses/sucrose ratio corresponds to decline in photosynthetic pigment degradation.

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