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Plastid pigments quantity and some physiological parameters related to photosyntetic processes in triticale grown for green biomass

H. Nedeva*, R. Ivanova, H. Yancheva

Crop Science Department, Faculty of Agronomy, Agricultural University, 12 Mendeleev, 4000 Plovdiv, Bulgaria

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Abstract. *The aim of the study was to establish the amount of plastid pigments and some physiological parameters related to photosynthetic processes in two triticale cultivars, grown for biomass under different nitrogen fertilization rates and harvesting stages in the region of Southern Bulgaria. The research was carried out in the field of Crop Science Department at the Agricultural University, Plovdiv, during the period 2013 – 2015. The experiment was set after the block method with four replications, and 20m^2 for each experimental plot. Two triticale cultivars – Musala and Attila grown for green biomass at different fertilizer rates (N_0, N_{120}, N_{160}, N_{200}, N_{240}) have been tested. The two cultivars are harvested in heading formation and milk maturity stages. The physiological parameters - intensity of transpiration, photosynthesis rate and the amount of plastid pigments (Chlorophyll A, Chlorophyll B, Carotene) have been observed at different cultivars and harvesting stages. In both tested cultivars in heading stage the transpiration intensity was higher when rates of N_{200} were used, while in milk maturity stage the highest values were in variant with N_{240}. The photosynthesis rate at both cultivars in heading stage was increased with increase of the nitrogen rate. In the stage of milk maturity the values of some indices decreased in both cultivars. The lowest content of plastid pigments in leaves of Musala cultivar was received in the control variant. It was observed that the increase of the nitrogen rate leads to increasing the pigments content. The highest content of pigments in Attila cultivar was received when N_{200} was applied.*

Keywords: triticale, green mass, fertilization, photosynthesis, pigments

Introduction

During the last years reduction of fossil raw materials altogether leads to our dependence on import and creates preconditions for searching different ways of obtaining energy. That results in finding opportunities to generate energy using renewable raw materials of plant origin. That's why the green biomass from corn, sorghum, rye, triticale, oats, etc. was increasingly used as a substrate for biogas production. Recent studies showed that triticale was the most suitable crop for Bulgarian ecological conditions. Triticale is a hybrid cereal developed by crossing wheat with rye. Over the past 20 years the interest in growing the crop was steadily increased due to its use as animal feed and bioenergy crop. Nearly 90% of the production of triticale in the world is concentrated in Europe, with about 7 million acres harvested per year.

With its high potential productivity, deeper root system, greater ecological plasticity, unpretentiousness to soil fertility, disease resistance, drought tolerance, increased tolerance to soil acidity, resistance to adverse soil and climatic conditions, the ability to grow as a short monoculture, and higher protein and lysine content in the grain and green mass, triticale has significant advantages over wheat, barley and maize. This was proved by the research of (Todorov, 2009; Pavlov and Kostov, 1984). Many studies showed that the rate and magnitude of the photosynthetic apparatus had significant effect on the formation of green biomass (Djatchouk et al., 2015; Janušauskaite and Feiziene, 2012; Hura et al., 2015).

The aim of the study was to establish the amount of plastid pigments and some physiological parameters related to photosynthetic processes in two triticale cultivars, grown for green mass in biogas production, under different nitrogen fertilization rates and harvesting stages in the region of Southern Bulgaria.

* e-mail: h_nedeva@abv.bg

Material and methods

The study was carried out in the field of Crop Science Department at the Agricultural University, Plovdiv, during the period 2013 – 2015. The experiment was set up following the block method with four replications, and 20m^2 for each experimental plot. Two triticale cultivars Musala and Attila, grown for green biomass under fertilizer rates of N_0, N_{120}, N_{160}, N_{200}, N_{240} have been studied. The biomass yield was reported in two harvesting stages – heading and milk maturity.

Physiological indicators as net photosynthesis speed (A), transpiration intensity (E) and stomata conductance (gs) were determined by portable photosynthetic system LCA-4 (Analitycal Development Company Ltd., Hoddesdon, England), together with leaf gas exchange, including net photosynthesis rate (A), transpiration intensity (E) and stomata conductance (gs).

All analyses for determination of plastid pigments content in mg/g – chlorophyll A, chlorophyll B, chlorophyll (A + B) and carotene were done in the laboratory of the Department of Physiology and Biochemistry at the Agricultural University- Plovdiv.

Triticale plants leaf area was defined by destructive method by taking linear measurements of leaf lamina and mathematical coefficient characteristic of a particular type (Berova et al, 2004) by the formula:

$$A = k.l.b,$$

where k is coefficient varying in different crops, l is length of the sheet

in the central vein, b is the maximum width of the sheet. The data was averaged for two-year periods – (2013/2014 and 2014/2015)

Statistical data processing was carried out in fourfold repetition. The experimental results were processed by one-way ANOVA, with the authenticity of differences determined by Student's t criterion at the following levels according to: ns – no evidence of difference; Gd = 5.0%; Gd = 1.0%; Gd = 0.1%.

Results and discussion

The results of our study showed that some physiological parameters of triticale differ among cultivars, fertilizer rates and harvesting stages. The data for flag leaf area (cm^2), length and width of flag leaf (cm) in the two harvesting stages (heading and milk maturity) are presented on Table 1. The results showed that the two studied cultivars differ regarding the area of assimilation (Kara, 2016). In heading stage the cultivar assimilation area varied from 25.46 cm^2 (Musala) up to 45.32 cm^2 (Attila) and in milk maturity stage from 18.17 cm^2 (Musala) up to 36.81 cm^2 (Attila). The average flag leaf area in both reporting stages was significantly lower in Musala cultivar compared to Attila cultivar. In both cultivars higher leaf area was reported in heading (29.89 cm^2, 41,71cm^2). In milk maturity stage leaf area slightly decreased (26.35 cm^2, 32.63 cm^2) mainly because of drying of some lower leaves.

According to the research of some authors (Rusakov, 1975; Shkurela, 1975; Pavlov and Kostov, 1984) nitrogen fertilization had the strongest influence on leaf area and its vital activity. They found that increase of nitrogen fertilizer rate in cereals leads to leaf index increase, photosynthetic potential and NSPFs and respectively dry matter increase per unit area. Our studies also showed a similar trend of increasing leaf area with increasing nitrogen fertilization rates, but to a certain extent (N_{200}), then its values declined. The highest values of assimilation area in both cultivars (Musala and Attila) in both stages were reported in application of N_{200}, but values in heading stage (33,37 cm^2, 45,32 cm^2) exceed those in milk maturity stage (29,57 cm^2, 36,81 cm^2).

The results presented in Table 2 indicated, that the harvesting stages for green biomass and various nitrogen fertilization rates lead to significant changes in indicators, defining leaf gas exchange. The reported rate of net photosynthesis in the heading and milk maturity at Musala exceed those at Attila cultivar. Net photosynthesis decreases with vegetation progressing. In both observed cultivars net photosynthesis was higher in heading stage compared to values in milk maturity. Regarding the effect of nitrogen rates on the net photosynthesis rate it was found that significantly higher intensity at both cultivars in heading stage had the application of N_{200} (21.98 $\mu mol\ m^{-2}\ s^{-1}$, 19,85 $\mu mol\ m^{-2}\ s^{-1}$).

The results for the transpiration intensity were too heterogeneous. In both harvesting stages higher transpiration intensity was reported at Atila cultivar. Lower values of the transpiration intensity were reported during heading, as the highest intensity was observed in the variant with N_{200} – 0.44 $mmol\ m^{-2}\ s^{-1}$ at Musala cultivar and 1.18 $mmol\ m^{-2}\ s^{-1}$ at Attila cultivar. During the next stages of plant growth as a result of increased average temperatures the transpiration intensity increased at both cultivars. In Mussala cultivar the values ranged from 0.90 $mmol\ m^{-2}\ s^{-1}$ when fertilized with N_{200} up to 1.34 $mmol\ m^{-2}\ s^{-1}$ when fertilized with N_{160}. The same tendency was noticed in Attila cultivar, where this indicator ranged from 0.92 $mmol\ m^{-2}\ s^{-1}$ at the zero fertilization option up to 1.79 $mmol\ m^{-2}\ s^{-1}$, at the variant fertilized with N_{160}. In both cultivars harvested in two stages, the stomata conductance was the lowest in control variant. In the variants with nitrogen application the differences between stomata conductance were insignificant (Table. 2).

Table 1. Leaf area indicators at the flag leaf in heading and milk maturity stages from central brother stem

Variants	Cultivars					
Fertilizer rates, кg/ha	Musala			Attila		
	Flag leaf length, cm	Flag leaf width, cm	Leaf area per flag leaf, cm^2	Flag leaf length, cm	Flag leaf width, cm	Leaf area per flag leaf, cm^2
	Heading stage					
N_0	21.72^c	1.86^c	25.46^c	29.09^c	2.04^b	37.35^c
N_{120}	23.50^b	2.03^b	30.07^b	31.51^b	2.11^b	41.8o^{ab}
N_{160}	26.09^a	1.90^c	31.22^b	31.95^{ab}	2.21^a	44.49^a
N_{200}	25.67^a	2.11^a	33.37^a	32.75^a	2.20^a	45.32^a
N_{240}	23.29^b	2.00^b	29.31^b	31.95^b	2.07^b	39.60^{bc}
120-240	24.05	2.18	29.89	31.15	2.13	41.71
	Milk maturity stage					
N_0	17.35^c	1.66^c	18.17^c	21.09^c	1.85^b	24.55^c
N_{120}	22.00^b	1.98^a	27.50^b	25.43^{bc}	1.95^b	31.26^b
N_{160}	24.40^a	1.88^b	28.92^a	26.24^b	2.16^a	35.76^a
N_{200}	23.90^a	1.96^a	29.57^a	27.09^a	2.15^a	36.81^a
N_{240}	22.05^b	1.99^a	27.60^b	26.27^b	2.12^a	35.08^{ab}
120-240	21.94	1.89	26.35	25.27	2.05	32.69

*Means within a column having a different superscript letter differ significantly ($p<0.05$)

Table 2. Gas exchange parameters

Fertilizer rates кg/ha	Net photosynthesis speed (A), μmol m^{-2} s^{-1}		Net photosynthesis intensity (E), mmol m^{-2} s^{-1}		Stomata conductance (gs), mmol m^{-2} s^{-1}	
	Heading	Milk maturity	Heading	Milk maturity	Heading	Milk maturity
			Musala – harvesting stages			
N_0	18.08^{c}	10.65^{b}	0.14^{e}	1.21^{b}	0.04^{b}	0.05^{c}
N_{120}	16.77^{d}	6.86^{e}	0.18^{d}	1.03^{c}	0.07^{a}	0.06^{b}
N_{160}	16.69^{d}	12.54^{a}	0.34^{s}	1.34^{a}	0.07^{a}	0.07^{a}
N_{200}	21.98^{a}	8.67^{d}	0.44^{a}	0.90^{d}	0.06^{a}	0.08^{a}
N_{240}	21.47^{b}	9.40^{c}	0.42^{b}	1.21^{b}	0.06^{a}	0.07^{a}
			Attila – harvesting stages			
N_0	16.66^{d}	9.80^{c}	0.97^{e}	0.92^{e}	0.04^{b}	0.05^{b}
N_{120}	17.26^{c}	9.94^{c}	1.05^{c}	1.32^{d}	0.06^{a}	0.07^{a}
N_{160}	16.49^{d}	11.70^{a}	1.12^{b}	1.79^{a}	0.07^{a}	0.07^{a}
N_{200}	19.85^{a}	10.67^{b}	1.18^{a}	1.38^{c}	0.06^{a}	0.06^{a}
N_{240}	19.02^{b}	11.43^{a}	1.01^{d}	1.58^{b}	0.06^{a}	0.06^{a}

*Means within a column having a different superscript letter differ significantly ($p<0.05$)

Table 3. Plastid pigments quantity (mg/g)

Fertilizer rates кg/ha	Chlorophyll A		Chlorophyll B		Chlorophyll (A+B)		Carotene	
	Heading	Milk maturity	Heading	Milk maturity	Heading	Milk maturity	Heading	Milk maturity
				Musala – harvesting stages				
N_0	1.80^{e}	1.31^{b}	0.85^{b}	0.48^{b}	2.65^{d}	1.79^{c}	0.67^{d}	0.61^{b}
N_{120}	2.02^{d}	1.54^{b}	0.89^{ab}	0.55^{b}	2.91^{c}	2.09^{b}	0.70^{d}	0.65^{b}
N_{160}	2.89^{a}	2.36^{a}	0.91^{ab}	0.83^{a}	3.80^{a}	3.19^{a}	1.09^{a}	0.90^{a}
N_{200}	2.75^{b}	2.28^{a}	0.98^{a}	0.87^{a}	3.73^{ab}	3.15^{a}	0.98^{b}	0.89^{a}
N_{240}	2.64^{c}	2.26^{a}	0.94^{ab}	0.86^{a}	3.58^{b}	3.12^{a}	0.89^{c}	0.88^{a}
				Attila – harvesting stages				
N_0	1.48^{c}	1.29^{b}	0.64^{c}	0.37^{c}	2.12^{c}	1.66^{d}	0.65^{b}	0.60^{c}
N_{120}	1.54^{b}	1.35^{b}	0.70^{bc}	0.41^{bc}	2.24^{c}	1.76^{c}	0.68^{b}	0.63^{bc}
N_{160}	1.66^{b}	1.48^{b}	0.76^{b}	0.42^{bc}	2.42^{b}	1.90^{b}	0.92^{a}	0.65^{b}
N_{200}	2.30^{a}	2.00^{a}	1.02^{a}	0.70^{a}	3.32^{a}	2.70^{a}	0.99^{a}	0.82^{a}
N_{240}	1.63^{c}	1.41^{b}	0.74^{bc}	0.48^{b}	2.37^{b}	1.89^{b}	0.63^{b}	0.59^{c}

*Means within a column having a different superscript letter differ significantly ($p<0.05$)

According to results of some researchers (Kaczmarczyk et al., 2013) the cultivars, harvesting stages and mineral fertilization had the significant influence on plastid pigments in triticale leaves. This was proved by our data (Table 3). The lowest plastid pigments content in leaves was reported in the variant without fertilization. Difference among the cultivars was observed, too. Cultivar Musala had higher content of plastid pigments compared to Attila cultivar. Plastid pigments changed with plant aging that leads to decrease of plastid pigments in leaves. It's more significant in chlorophyll and less in carotenoids, especially in variants with higher nitrogen rates. In Mussala cultivars plastid pigments content in leaves (chlorophyll A, chlorophyll B and carotene) was increased by increasing the fertilization rates up to N_{160}, then their content slightly decreased with exception of chlorophyll content that was the highest in variant with N_{200}. In Attila cultivars plastid pigments content in leaves was increased by increasing the fertilizer rates. The highest values of all plastid pigments were reported when fertilizer rate of N_{200} was used.

Conclusion

The studied cultivars Musala and Attila had different physiological charactersitics and plastid pigments as a response to harvesting stages and nitrogen fertilization rates. Cultivar Musala

had significantly lower leaf area compared to Attila cultivar in both harvested stages but in heading stage the leaf area had the higher value in both cultivars. The net photosynthesis intensity and plastid pigments of cultivar Musala exceeded this recorded in Attila in both stages but had lower transpiration intensity. Photosynthesis intensity and plastid pigments decreased with plant aging while transpiration intensity increased for both cultivars. The highest net photosynthesis for both cultivars was observed in heading stage and N_{200} application.

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CONTENTS 1 / 2

CONTENTS 2 / 2

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