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RESEARCH ARTICLE

Nitrogen assimilation and photosynthetic capacity of wheat genotypes under optimal and deficient nitrogen supply

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Abstract The performance of two contrasting Bulgarian wheat varieties (Slomer, an old tall cultivar, and Enola, a modern semi-dwarf one) to nitrogen deficiency was compared by measuring biochemical parameters characterizing N uptake and assimilation as well as growth and photosynthetic activity of young seedlings. The old genotype displayed better photosynthetic capacity, higher N assimilation expressed by elevated amino acid synthesis and better overall performance under N limitation. This could be explained by the fact that selection of old varieties was performed mostly in environments with low nutrient availability and consequently these genotypes proved to be more suitable for growing on low-input conditions. Upon limiting N supply modern variety preferentially accumulated sugars while the old one retained higher amino acids levels. It was demonstrated that processes involved in N metabolism were tightly interrelated with photochemical reactions and carbon assimilation even at early developmental stage.

Keywords Chlorophyll fluorescence · Glutamine synthetase · Glycolate oxidase · Nitrate reductase · Nitrogen use efficiency · *Triticum aestivum*

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Introduction

Nitrogen is a major macro-nutrient essential for plant growth and development and is a constituent of most cellular components such as amino acids, proteins, nucleic acids, chlorophyll, and many other metabolites and hormones (Krapp 2015). Nitrogen content has an important role in crop yield, and is therefore used as a measure for crop productivity in field conditions. Plants abilities to utilize nitrogen from the soil are defined by the so-called nitrogen use efficiency (NUE) which represents the ratio of grain yield to the amount of applied or available nitrogen and generally is not a very high value (Dobermann 2005; Krapp 2015). Physiological NUE can be expressed as the fresh or dry mass produced per unit nitrogen content in the plant and depends on the absorption efficiency (amount of absorbed N/quantity available N) and utilization efficiency (yield/absorbed N) (Hirel et al. 2011; Perchlik and Tegeder 2018). It is generally accepted that NUE in cereals has a relatively low value, since only about 33% of N fertilizers applied globally are recovered in the grain (Hawkesford and Griffiths 2019). The improvement of NUE is of substantial importance for yield management of economically valuable crops. Lowering nitrogen fertilizer use and breeding plants with higher NUE is one of the main challenges of sustainable agriculture and environmental safety (Masclaux-Daubresse et al. 2010). Moreover, it is estimated that the demands for nitrogen fertilizers are increasing with the increasing needs to feed the constantly growing human population (Hirel et al. 2011).

Wheat is among the main cereals cultivated mostly for human consumption world-wide and represents a major crop for Bulgarian agriculture in particular. Since wheat is grown with preference for its grain protein content it requires large quantities of inorganic N fertilizers, the

excess application of which among others has harmful impact on the environment (Kichey et al. 2006; Chandna et al. 2012).

In order to select genotypes with potential for more efficient nitrogen utilization it is important to study the uptake and assimilation pathways of nitrogen in wheat varieties which would be a useful step towards improving yield and economic investment, minimizing the losses of fertilization (Krapp 2015; Pan et al. 2020).

For most plants, except legumes, inorganic N is available in the form of nitrate or ammonium (Kichey et al. 2006; Masclaux-Daubresse et al. 2010; Bloom 2015). After being absorbed from the soil by roots, nitrate is transported to plant cells via specific transport proteins. In leaf cytosol nitrate is converted to nitrite by the enzyme nitrate reductase (NR) using energy and reductant (NADH, NADPH) from photosynthesis and respiration of green tissues (Bloom 2015). The resulting product, nitrite, is further reduced to ammonium by nitrite reductase (NiR) in chloroplasts or plastids. The produced ammonium is incorporated into the amino acid glutamate through the action of glutamine synthetase (GS) in conjunction with glutamate synthase also known as glutamine oxoglutarate aminotransferase (GOGAT). Ammonium generated via photorespiration and protein degradation is also reassimilated by the GS/GOGAT cycle. Besides substrates, photorespiration provides also reducing equivalents for nitrogen assimilation and particularly for nitrate reduction and on the other hand, it contributes to the synthesis of several amino acids (Bauwe et al. 2010; Bloom 2015; Zivcak et al. 2015). Furthermore, N assimilation requires reduced compounds such as NADH and NADPH and is a highly energy requiring process. ATP produced in mitochondrial oxidative metabolism, photosynthesis and photorespiration are thus tightly linked with the processes of chlorophyll fluorescence and carbon metabolism with N assimilation which could be a key regulatory target for improving plant productivity under limiting conditions.

Thus, a more profound understanding of N metabolism and its regulation in combination with various approaches for achieving higher NUE in crops and exploring the natural variability of plant performance under high- and low-N environments would be beneficial for improving plant productivity (Krapp 2015).

In a previous study we have found that old Bulgarian wheat variety Slomer had higher N-efficiency when grown on poor soil with limited N supply (under more extensive conditions) compared to its efficiency on rich soils with higher N content (Landjeva et al. 2014). On the contrary, modern Bulgarian wheat genotype Enola proved to be inefficient on poor soils, while more efficient on rich ones. This could be explained by the fact that selection of old varieties was performed mostly in environments with low nutrient availability and consequently these genotypes proved to be more suitable for growing on low-input conditions.

Here, we have followed the changes in leaf metabolite concentrations and enzyme activities involved in N metabolism, as well as growth and photosynthetic activity of both genotypes at critical seedling stage in order to provide a better understanding of their mode of N management under control and nitrogen deficiency environments.

The aim of the present work was to study the impact of N supply on physiological processes and some functional aspects of N nutrition and photosynthesis by comparing the reaction of contrasting wheat varieties differing in their response to various N levels in field conditions. In view of the above, some of the studied parameters could be proposed as markers in modelling approaches or as selection criteria for improving NUE in breeding programs.

Materials and methods

Plant material and growing conditions

Two Bulgarian bread wheat varieties, Slomer and Enola, with contrasting nitrogen use efficiency (NUE) were used in the experiments. Slomer is an old tall variety which exhibited irresponsiveness to nitrogen supply in field conditions (Landjeva et al. 2014) but had better adaptability to N deficiency, contributing to its higher NUE. Enola is a modern semi-dwarf variety with higher agronomic efficiency towards nitrogen assimilation and its allocation to the grain.

After surface sterilization (5 min soaking in 0.1% sodium hypochlorite) seeds were germinated on wet filter paper in Petri dishes at 25 °C in darkness for 48 h. Under normal conditions (control nitrogen concentration) seedlings were transferred to nutrient solution with the following macronutrients: 2.5 mM $Ca(NO_3)_2.4H_2O$, 2.5 mM KNO_3, 2 mM $MgSO_4.7H_2O$, 1 mM KH_2PO_4 or a total of 7.5 mM nitrates. For nitrogen deficiency conditions nitrogen levels were reduced to 1/10th of controls: 0.25 mM $Ca(NO_3)_2.4H_2O$ and 0.25 mM KNO_3 giving an overall of 0.75 mM of nitrates, while all other nutrient components remained as in controls. Micronutrients composition was equal for both variants: 9 μM Fe-EDTA, 4.0 μM H_3BO_4, 1.0 μM $CuSO_4$, 0.9 μM $ZnSO_4$, 1.8 μM $MnCl_2$, 0.2 μM $NaMoO_4$, Young plants/seedlings were grown in a climatic chamber with 22/18 °C day/night temperature respectively, 12 h photoperiod, irradiance of 250 μmol m^{-2} s^{-1} and 70% relative humidity for 14 days (until they reached the stage of second fully developed leaf).

Plant biometry

For the experiments, 10 plants of each variant were collected and root and shoot length as well as fresh weights were measured using analytical balance (KERN ABJ, Balingen) and a metric ruler.

Measurement of free amino acids, total soluble sugars and total soluble protein

Freshly harvested samples of 1 g leaf tissue were stirred in 5 ml 95% ethanol. The insoluble fraction of extracts was rinsed three times with 5 ml 70% ethanol. After 5 min centrifugation at 4500×*g* supernatants were collected and evaporated to dryness. They were then dissolved in 1 ml distilled water and used for analysis of amino acids and sugars. Total content of free amino acids was determined according to the ninhydrin method of Yemm and Cocking (1955), extinction was detected at 580 nm (on UV/VIS Spectrophotometer, PG Instruments Ltd.) and concentration was estimated from a standard curve with L-leucine as standard. Soluble sugars were assessed by the phenol-sulfuric acid procedure described by Ashwell (1966). Extinction was read spectrophotometrically at 490 nm and concentration of soluble sugars was calculated from a calibration curve using sucrose as standard. Total soluble protein content in fresh leaves was estimated according to Bradford (1976) using bovine serum albumin as standard.

Nitrate content in leaves was assessed by the salicylic acid method of Cataldo et al. (1975). Tissue was homogenized in 100 mM phosphate buffer pH 7.4 (1 g FW in 6 mL buffer), centrifuged for 15 min at 30,000×*g* and 0.2 ml supernatant aliquots was reacted with 0.8 ml of 5% salicylic acid in concentrated H_2SO_4. After 20 min incubation at room temperature, 19 ml of 2N NaOH was added and absorbance was read at 410 nm. Nitrate concentration was determined from a previously prepared standard curve and expressed as nmol per g FW.

Nitrogen content in leaves was determined by the method of Kjeldahl digestion with sulfuric acid using Velp UDK159 automatic Kjeldahl analyzer following the protocol described in the manual.

Enzyme activities

Enzyme activities in leaves were measured by in vitro methods. For nitrate reductase (NR, EC 1.7.1.1) and glutamine synthetase (GS, EC 6.3.1.2) activity 0.5 g fresh leaf tissue was ground to a fine powder in pre-chilled mortar with liquid N_2. The extraction buffer (pH 8.0) contained 50 mM Tris HCl, 10 mM $MgSO_4$, 1 mM EDTA, 10 mM cysteine, 1% insoluble PVP. The buffer was added to the tissue powder in proportion 2.5 ml to 0.5 g. The homogenate was centrifuged at 4 °C for 15 min at 12,000×*g*. Enzyme activity was measured in the supernatant.

The method of Hageman and Hucklesby (1971) for NR activity was used with optimization for barley plants as described by Lillo (1983). The reaction mixture comprised of 100 mM phosphate buffer (pH 7.4), 1 mM EDTA, 10 mM KNO_3, 10 mM cysteine, 0.4 mM NADH and 0.2 ml enzyme extract. The reaction was terminated after 10 min of incubation at 30 °C by the addition of 0.2 ml 0.1 M Ba acetate and centrifuged at 7000×*g* for 15 min. The amount of nitrites produced was measured spectrophotometrically at 540 nm in the supernatant after addition of 1% sulfanilamide in 1.5 N HCl and 0.2% N-(1-naphtyl)-ethylenediamine dihydrochloride.

GS activity was measured according to O'Neal and Joy (1973). Reaction mixture contained 50 mM Tris-HCl, 50 mM $NH_2OH.HCl$, 80 mM glutamate, 20 mM $MgSO_4$, 8 mM ATP, 1 mM EDTA and 0.5 ml enzyme extract. After 10 min of incubation at 30 °C the mixture was centrifuged at 5000×*g* for 15 min. The reaction was terminated by the addition of 0.75 ml of solution containing equal quantities of 10% $FeCl_3$ dissolved in 0.2 N HCl, 24% Trichloroacetic acid and 17.5% HCl. Extinction was read spectrophotometrically at 540 nm.

Glycolate oxidase (GO, E.C. 1.1.3.15) activity was assayed according to Baker and Tolbert (1966) following the formation of glyoxylate phenylhydrazone. Leaf tissue (0.5 g) was ground at 0 °C with extraction mixture containing 100 mM phosphate buffer (pH 8.0), 1 mM EDTA and 10 mM cysteine. Reaction mixture contained 300 µl leaf extract, 100 µl extraction buffer, 50 µl 100 mM potassium glycolate, 1.6% phenylhydrazine hydrochloride and 100 µl freshly prepared flavin mononucleotide (4.8 mg FMN in 10 ml H_2O) and water to a final volume of 1.5 ml. After 15 min of incubation at 30 °C the reaction was stopped by adding 500 µl of 10 N HCl. Color development was induced by the addition of 100 µl 8% $K_3Fe(CN)_6$ and *A535* was determined spectrophotometrically against a blank sample without glycolate. Under these conditions in a 1-cm cuvette, 0.01 µmol of glyoxylate phenylhydrazone gave an *A535* of 0.186 (Zelitch et al. 2009).

Chlorophyll fluorescence measurements

Prompt chlorophyll fluorescence was measured from the middle part of the first leaf, after exclusion of the leaf tip and leaf base, by a pulse modulation chlorophyll fluorometer (PAM 101; H. Walz' Effeltrich' Germany) using actinic light at 330 µmol m^{-2} s^{-1} and saturating light at 3500 µmol m^{-2} s^{-1} photon flux density. The minimum chlorophyll fluorescence yield in the dark-adapted state and in the light-adapted state (F_0 and F_0', respectively),

maximum chlorophyll fluorescence yield in the dark-adapted state and in the light-adapted state (F_m and F_m', respectively), and steady-state chlorophyll fluorescence (F_s) were recorded. The following parameters were calculated according to Roháček (2002): maximum variable chlorophyll fluorescence yield in the dark-adapted state ($F_v = F_m - F_0$); potential maximum quantum yield of PSII (F_v/F_m); F_v/F_0; actual quantum yield of PSII [$\Phi_{PSII} = (F_m' - F_s)/F_m'$]; effective quantum yield of PSII photochemistry [$\Phi_{exc} = (F_m' - F_0')/F_m'$]; non-photochemical chlorophyll fluorescence quenching [$NPQ = (F_m - F_m')/F_m'$]. Coefficient of photochemical quenching (q_L) that measures the fraction of open PSII centers based on a lake model for the PSII photosynthetic apparatus, quantum yield of dissipation by down regulation in PSII (Φ_{NPQ}) and quantum yield of non-regulated energy dissipated in PSII (Φ_{NO}) were calculated according to Kramer et al. (2004).

$$\Phi_{NPQ} = 1 - \Phi_{PSII} - \frac{1}{NPQ + 1 + q_L\left(\frac{F_m}{F_0} - 1\right)};\ \Phi_{NO} = \frac{1}{NPQ + 1 + q_L\left(\frac{F_m}{F_0} - 1\right)}$$

Statistical analysis

Three independent experiments were set and all parameters were measured in at least three replicates each time (unless otherwise stated in the figures). Data was analyzed by nonparametric statistical methods using the software package STATISTICA 7 (StatSoft 2005). To ascertain the effect of genotype at optimal and deficient N supply, as well as the effect of N level for each genotype, one-way Kruskal–Wallis test was used at overall significance level of 0.05. To assess differences in the pair-wise comparisons (1) at optimal and deficient N level and (2) in the modern and old cultivar, Mann-Whitney U-test was run at overall significance level $P < 0.05$.

Results

Plant biometry

Significant differences were observed between the two varieties grown under sufficient nitrogen concerning shoot biomass accumulation (assessed by fresh weight measurement, Fig. 1a), with higher values in Enola compared to Slomer controls. However, low N supply did not lead to considerable changes in this parameter. Likewise, root biomass and plant shoot length did not show substantial differences between genotypes and different nitrogen supply (Fig. 1b, c). Root length did not vary in Enola between the two nitrogen levels, but Slomer had longer roots under low nitrogen supply compared to control (Fig. 1d).

Biochemical analyses

Enola exhibited comparatively greater amount of sugars than Slomer under control N nutrition, which was drastically increased after N deficiency, making the difference between the two varieties rather profound. Compared to Slomer, Enola had twice as high sugars in the leaves upon N deficiency (Fig. 2a).

An opposite tendency was observed regarding total amino acid content in the leaves of two varieties. Under control N supply Slomer had more than 4 times greater amount of amino acids in leaves compared to Enola. Upon N insufficiency both varieties showed distinctive decrease in amino acids content expressed as 2.78-fold reduction in Enola and only 1.65-fold reduction for Slomer. Under N starvation Slomer preserved nearly 7 times greater amino acid amounts than Enola which was as much as 2.5-fold greater even compared to Enola control levels (Fig. 2b).

No significant changes between control and low N supply were found regarding total N and protein content in the leaves of both studied varieties (Fig. 2c, d).

Under optimal N supply Slomer had 2-fold higher nitrates content in the leaves but N deprivation caused a significant decrease of NO_3^- concentrations in both genotypes (Fig. 3b).

Enzyme activities

NR activity and leaf nitrate levels in both cultivars were higher when plants were supplied with control N doses and decreased with the reduction of N in the nutrient solution (Fig. 3a, b). Under normal N supply (control) Enola had 35% higher NR activity than Slomer but a corresponding 50% lower nitrate content in Enola compared to Slomer leaves. NR activity dramatically dropped upon N deprivation and greater reduction with respect to controls was observed in Enola. Still, similar nitrate content in the leaves was measured in the two varieties under low N.

Regarding GO, Slomer had higher activity than Enola when the two were grown on sufficient N (Fig. 3c). Upon N deficiency, GO activity decreased in both varieties, with Slomer retaining significantly higher GO activity than Enola.

GS activity under sufficient N was higher in Enola than Slomer. Nevertheless, N limitation caused a pronounced decrease in Enola, while Slomer maintained its GS activity at control values (Fig. 3d).

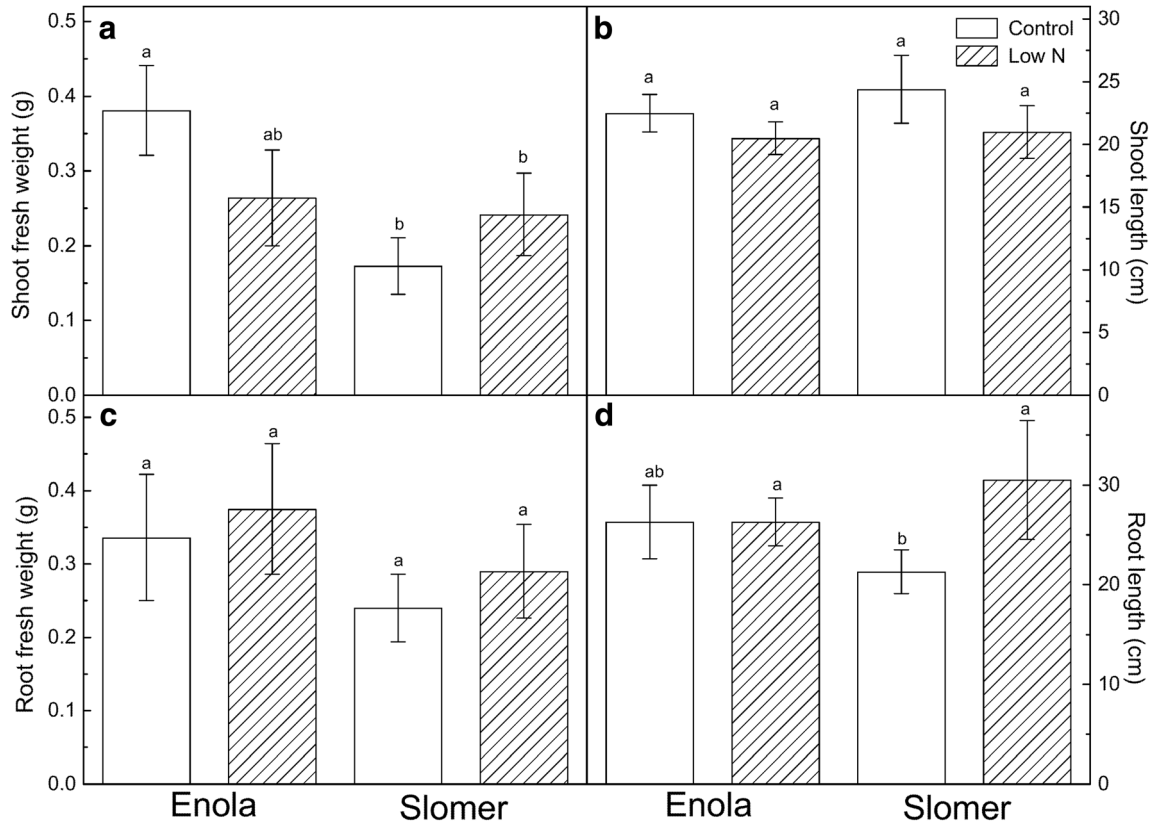

Fig. 1 Plant biometry of two Bulgarian wheat varieties (Enola and Slomer) grown under control and low N conditions. (**a**) Shoot fresh weight; (**b**) Shoot length; (**c**) Root fresh weight; (**d**) Root length. Data is presented as means ± SE (n = 10). Different letters indicate significant differences in the pair-wise comparisons at optimal and deficient N levels, and within both cultivars at $P < 0.05$ according to Mann-Whitney U-test

Chlorophyll fluorescence parameters

Upon sufficient N supply Slomer had higher F_v/F_0 ratio which is considered a sensitive indicator of maximal efficiency of photochemical processes in PSII and higher potential quantum yield of PS II (F_v/F_m). N limitation did not induce changes in quantum yields of photochemical to non-photochemical processes in PSII in either variety (Fig. 4a, b).

Non-photochemical quenching (NPQ) was not significantly different between the two genotypes under control conditions, but upon nitrogen deficiency it was reduced in Slomer and did not vary in Enola (Fig. 4c). Under control nitrogen supply yield decrease of open reaction centers, expressed by Φ_{exc} was greater in Slomer compared to Enola. Upon N limitation Φ_{exc} remained unchanged in Enola, but increased slightly in Slomer (Fig. 4d).

The quantum yield of regulated non-photochemical energy loss in PSII, assessed by Φ_{NPQ}, was higher in Enola than Slomer at normal nitrogen supply levels. Upon N limitation Φ_{NPQ} remained at control values in Enola and was reduced in Slomer (Fig. 5a).

The quantum yield of non-regulated energy loss in PSII assessed by Φ_{NO} displayed no significant differences between the two genotypes and was not influenced by N deficiency (Fig. 5b).

The effective quantum yield of photochemical energy conversion, estimated by Φ_{PSII} was higher in Slomer. However, limited N did not induce changes in this parameter in either variety (Fig. 5c).

Discussion

Native wheat varieties are important with their representing rich genetic resources which could confer better adaptability to various abiotic stresses (Krapp 2015). Generally, modern wheat genotypes are highly productive but also susceptible to insufficient nutrient supply in the soil (Dobermann 2005; Chandna et al. 2012; Landjeva et al. 2014).

This study compared the reaction of two Bulgarian wheat varieties (Slomer, an old tall cultivar, and Enola, a modern semi-dwarf one) to nitrogen deficiency by measuring biochemical parameters characterizing the uptake

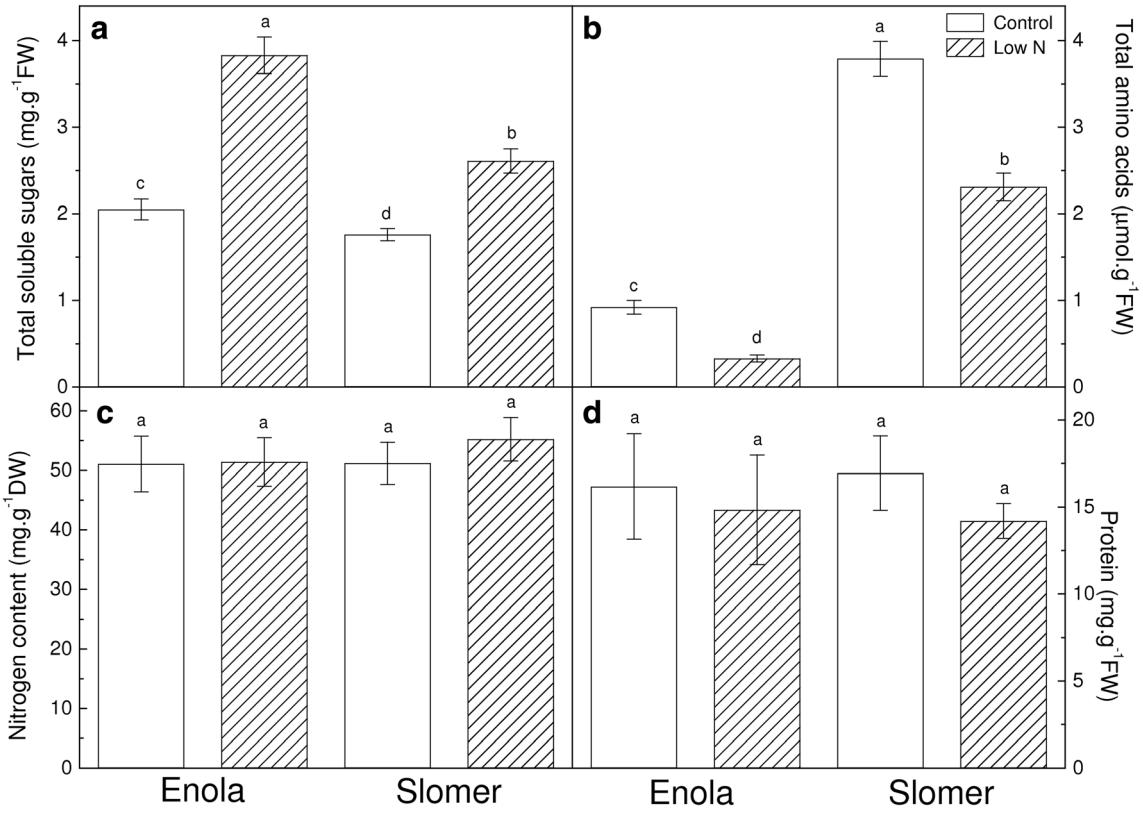

Fig. 2 Leaf content of soluble sugars (**a**), total amino acids (**b**), nitrogen (**c**), and soluble protein (**d**) in two Bulgarian wheat varieties under control and low N conditions. Values are means ± SE ($n = 6$). Different letters indicate significant differences in the pair-wise comparisons at optimal and deficient N levels, and within both cultivars at $P < 0.05$ according to Mann-Whitney U-test

and assimilation of nitrogen at early seedling stage. In a previous study we have shown that the chosen genotypes displayed contrasting behavior regarding yield and certain agronomic parameters when grown on various nitrogen supplies in the field (Landjeva et al. 2014). Obtained results encouraged us to extend the study towards better understanding of the mechanisms of nitrogen uptake and its incorporation into amino acids and proteins and also to evaluate the functional state of the photosynthetic apparatus at early developmental phase.

NUE is a complex trait dependent on multiple physiological processes, including N uptake, assimilation, allocation, and remobilization and it is usually regarded as comprising two major components, nitrogen uptake efficiency (NUpE) and nitrogen utilization efficiency (NUtE) (Perchlik and Tegeder 2018). Crops with high NUpE that grow well at low N are of particular importance for sustainable agriculture. A recent study found that modern wheat cultivars had significantly higher root NUpE than old ones and this genotypic variation was characterized as a stable and adaptive trait (Zhang et al. 2020). Moreover, higher N deficiency tolerance was associated with higher N assimilation efficiency and adaptive growth strategy in novel hexaploid wheat (Yang et al. 2018). Using spring barley genotypes and comparing field and laboratory data, Beatty et al. (2010) demonstrated that the contribution of N uptake (NUpE) and N utilization (NUtE) to the overall NUE depending on the level of supplied N. Although similar NUE were estimated for both environments, hydroponically grown plants showed a decrease in N uptake at higher N supply levels, while the opposite N uptake efficiencies were observed in the field.

Nitrogen assimilation is especially important for plants growth, development and adaptability to environmental stresses and also with its close relationship to photosynthesis, photorespiration and secondary metabolism (Yang et al. 2018). In our experiments plant growth was not affected by lower nitrogen supply, which indicated a considerable tolerance towards nitrogen insufficiency under controlled conditions and at early developmental stage. The absence of significant changes in soluble protein content in the leaves implied protein degradation was not the cause for observed variation in amino acid pool but it was rather the result of de novo synthesis.

In plants, free NO_3^- represent up to 50% of the total nitrogen and may function as favorable osmotic compound supporting ion homeostasis and helping maintain cellular water status along with other osmotically active substances

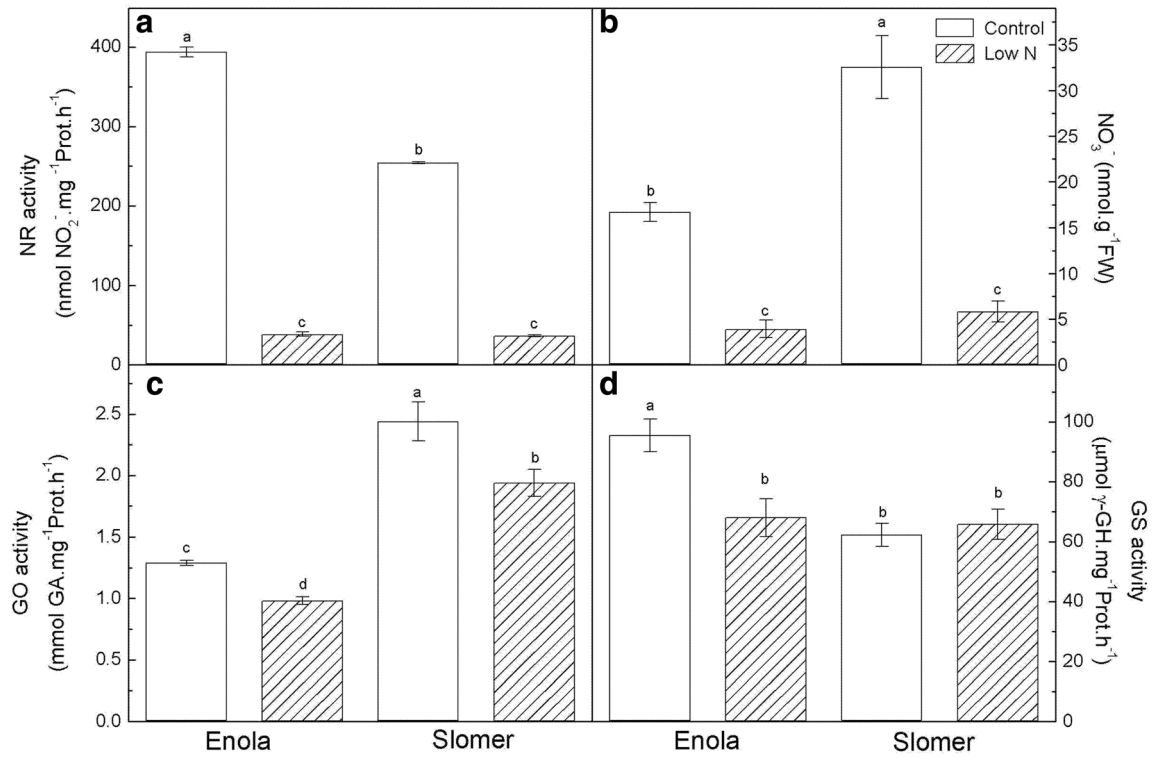

Fig. 3 Nitrate reductase (NR) activity (**a**), nitrate content (**b**), Glycolate oxidase (GO) activity (**c**), and Glutamine synthetase (GS) activity (**d**) in leaves of two Bulgarian wheat varieties grown under control and low N conditions. Values are means ± SE ($n = 6$). Different letters indicate significant differences in the pair-wise comparisons at optimal and deficient N levels, and within both cultivars at $P < 0.05$ according to Mann-Whitney U-test

such as soluble sugars and free amino acids (Iqbal et al. 2016; Blum 2017). Additionally, higher nitrate in guard cells contribute to stomata opening which is advantageous to gas exchange and stimulates C assimilation and photosynthetic processes in general. Our results support the above-mentioned tendencies by evidencing that the genotype with better photosynthetic performance (old variety Slomer) also exhibited higher amount of osmotically active substances and greater activity of GO, a principal enzyme of photorespiration pathway. It was documented that increased GO activity could be beneficial under stressful environments (Cui et al. 2016). Peroxisomal enzyme GO uses oxygen to catalyze the oxidation of glycolate into glyoxylate and H_2O_2. Further transformation of glyoxylate into serine generates NADH and NH_4^+ which could be donated to the processes of nitrate reduction and ammonium assimilation respectively. Thus, photorespiration and nitrate assimilation represent important crossovers of plant carbon and nitrogen metabolism (Bauwe et al. 2010; Bloom 2015; Krapp 2015). Moreover, photorespiration provides substrates for other biochemical pathways and influences fundamental primary metabolic routes such as photosynthesis, nitrate assimilation and the Krebs (or tricarboxylic acid) cycle (Peterhansel et al. 2010; Bloom 2015; Dellero et al. 2016). Directing ammonium produced in photorespiration towards amino acid pools could eventually contribute to the improvement of plant NUE.

The first step of nitrogen assimilation is controlled by the enzyme NR which reduces nitrates to nitrites followed by the stoichiometric reduction of nitrites to ammonium which is subsequently incorporated into amino acids predominantly via the GS-GOGAT cycle. Afterward, GS catalyzes the re-assimilation of ammonia but it is also a rate-limiting step of photorespiration processes and is a key enzyme engaged in managing plant productivity (Kichey et al. 2006; Masclaux-Daubresse et al. 2010; Zivcak et al. 2015). Therefore, GS represents a main connection point between N and C assimilation and its regulation is considered strategically important for plant homeostasis (Nemeth et al. 2018). Some authors consider the activity of GS together with total N, chlorophyll, and soluble protein as indicators for N assimilation and recycling (Zhong et al. 2018). Recently, Iqbal et al. (2020) presented strong evidence that variations in N metabolism were tightly associated with NUE in cotton genotypes.

According to Chandna et al. (2012) the recognition or discovery of genotypes with high nitrogen efficiency which also performed well under limiting N conditions would provide candidates for use in sustainable farming and reduction of nitrate contamination in the environment. The

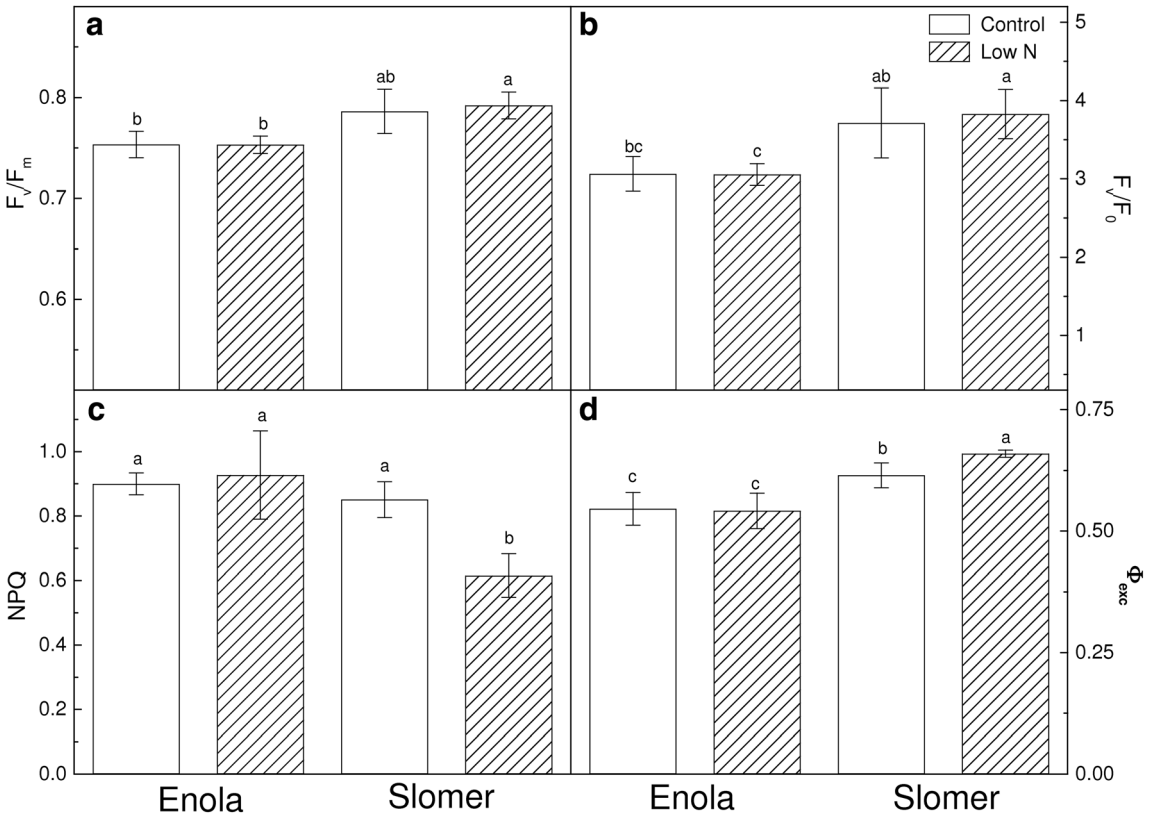

Fig. 4 Chlorophyll fluorescence parameters measured in leaves of two Bulgarian wheat varieties grown under control and low N conditions. (**a**) Maximum quantum yield of PSII (F_v/F_m); (**b**) Maximum efficiency of photochemical processes in PS II and/or potential photosynthetic activity (F_v/F_0); (**c**) Non-photochemical chlorophyll fluorescence quenching (NPQ); (**d**) Effective quantum yield of PS II (Φ_{exc}). Values are means ± SE (n = 10). Different letters indicate significant differences in the pair-wise comparisons at optimal and deficient N levels, and within both cultivars at $P < 0.05$ according to Mann-Whitney U-test

old wheat variety Slomer used in our study had greater nitrate content compared to modern Enola correlating with sustained higher levels of amino acids in the leaves which could be indicative of higher uptake efficiency under limiting N supply. Slomer also exhibited higher effective quantum yield photochemical energy conversion in PS II (Φ_{PSII}) which is in agreement with the work of Perchlik and Tegeder (2018) who found that higher amino acids corresponded with better photosynthetic activity and higher PNUE (the proportion of leaf N used for C fixation per unit leaf area) in Arabidopsis. It is known that high doses of N stimulate photosynthetic performance and NUE possibly through redistribution of N towards Rubisco protein (Seemann et al. 1987). Our experiments we have revealed that N supply only slightly affected photochemical processes as assessed by chlorophyll a fluorescence analysis. Interestingly, upon N limitation Slomer had the best performance expressed by lower photoinhibition through thermal dissipation of excitation energy (NPQ) and increased effective quantum yield of PSII photochemistry (Φ_{exc}).

The examined wheat genotypes also differed in their preference of leaf osmolytes: the old variety Slomer exhibited significantly higher amounts of N containing amino acids and nitrate ions, while the modern Enola favored soluble sugars as osmotics, especially during N insufficiency. Obviously, under optimal N supply Slomer more actively accumulated nitrates and allocated them to the leaves (higher NUpE), thus when subjected to N deficiency, Slomer displayed higher amino acid levels compared to Enola (higher NUtE as well).

Genetic effects on NUE should also be considered. Previous studies on US and European wheat germplasm demonstrated that semi-dwarf stature significantly affected NUE related parameters. Thus, varieties released after 1960s (a landmark defined by the introduction of semi-dwarf varieties) showed significant trend for improved NUpE, NUtE, nitrogen harvest index and other NUE traits (Guttieri et al. 2017). Using a set of near-isogenic lines for various semi-dwarfing genes (*Rht* genes), Gooding et al. (2012) demonstrated that adding a single gene to a tall genetic background reduced NUE in organic production system (i.e. in conditions of nitrogen deficiency). Recently, a role of *Rht* genes was also suggested in protection of the photosynthetic apparatus under heavy metal stress

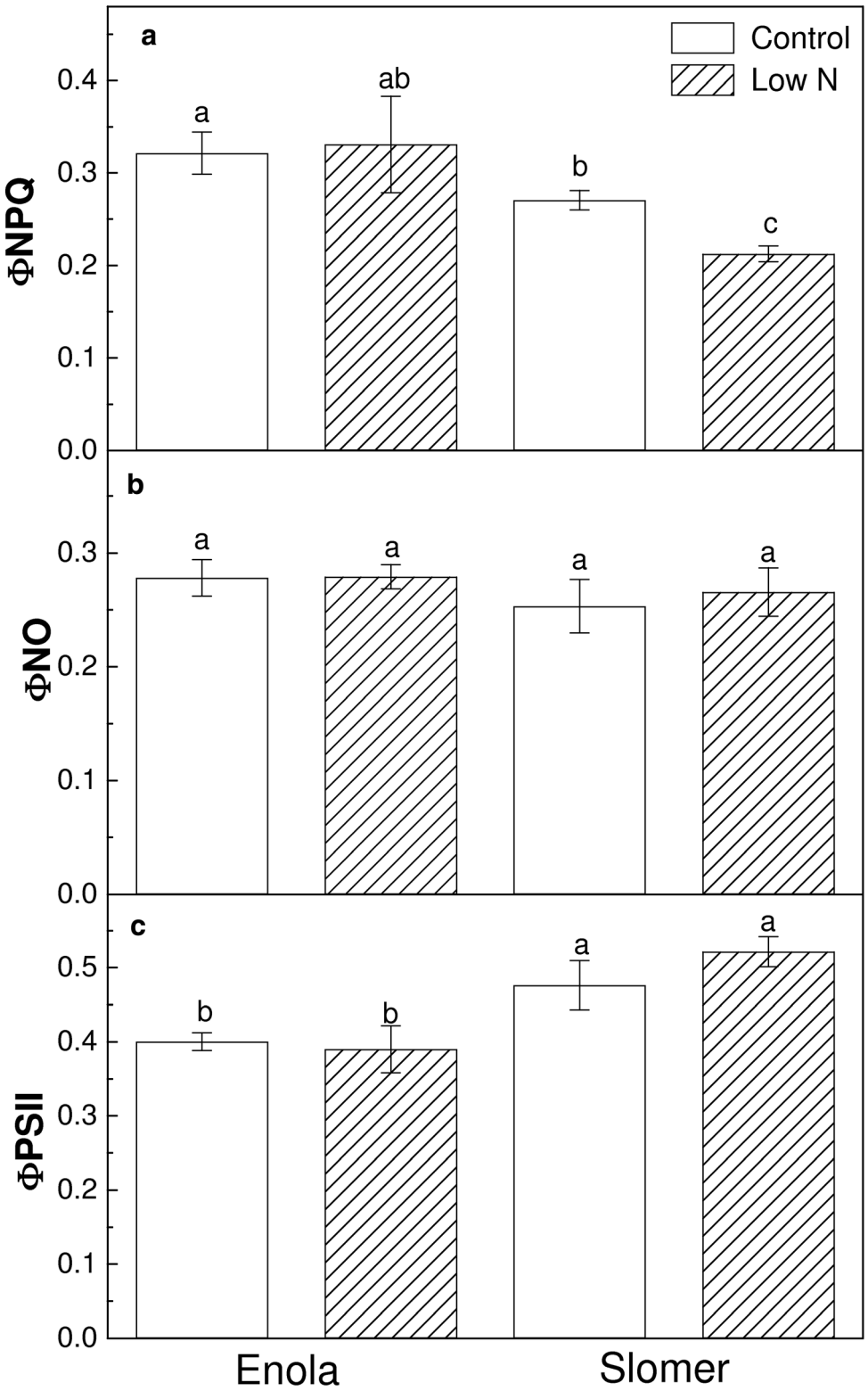

Fig. 5 Quantum yields of dissipative processes for the energy absorbed by PSII. (**a**) Yield of regulated non-photochemical energy loss in PSII (Φ_{NPQ}); (**b**) yield of non-regulated energy dissipation (Φ_{NO}); (**c**) effective quantum yield of photochemical energy conversion in PSII (Φ_{PSII}) measured in leaves of two Bulgarian wheat varieties grown under control and low N conditions. Values are means ± SE (n = 10). Different letters indicate significant differences in the pair-wise comparisons at optimal and deficient N levels, and within both cultivars at $P < 0.05$ according to Mann-Whitney U-test

(Dobrikova et al. 2017). The contemporary variety Enola carries two *Rht* genes—*Rht-B1b/d* and *Rht8* (Landjeva et al. 2011), while Slomer is an old local variety of tall stature carrying wild alleles at both *Rht* loci.

Conclusions

Wheat genotypes with contrasting N-use-efficiency in field conditions displayed considerable variation in photosynthetic capacity, enzyme activities and osmolyte concentration in response to different N supply. It was demonstrated that consequences of limiting N nutrition were interrelated with photosynthetic metabolism. Nitrate reduction and N assimilation are important energy and C substrate consuming pathways in leaves and perturbation of these processes could disclose tight coordination and interplay between photochemical reactions and carbon metabolism.

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