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ORIGINAL ARTICLE

Nitrogen-induced variations in leaf gas exchange of spring triticale under field conditions

Daiva Janusauskaite[1] · Dalia Feiziene[1] · Virginijus Feiza[2]

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Abstract Nitrogen (N) is the key factor limiting photosynthetic processes and crop yield. Little is known about the response of leaf gas exchange of spring triticale (*Triticosecale* Wittm.) to N supply. The effect of N fertilizers on different gas exchange variables, i.e., photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s), instantaneous water use efficiency (WUE) and maximum quantum yield of photosystem II (PSII) (F_v/F_m), chlorophyll index (SPAD, soil–plant analysis development), and the relationship of these variables with yield were studied in spring triticale grown under field conditions. Six treatments of N—0, 90, 180, 90 + 30, 90 + 30 + 30 kg ha^{-1} (applied as ammonium nitrate, AN) and one treatment of N 90 + 30 + 30 kg ha^{-1} (applied as urea ammonium nitrate solution, UAN) were compared. The analysis of variance showed that throughout the triticale growing season, N fertilization had significant effects on A, WUE, g_s and SPAD. On average, N fertilizer application increased A values by 14–70%. E and F_v/F_m values were not influenced by N fertilization levels. The effect of growth stage and year on gas exchange variables and F_v/F_m and SPAD was found to be significant. At different growth stages, A values varied and maximum ones were reached at BBCH 31–33 (decimal code system of growth stages) and BBCH 59. With aging, values of A decreased independently of N fertilization level. The gas exchange variables were equally affected by both fertilizer forms. The interplay among grain yield, leaf gas exchange variables, F_v/F_m and SPAD of spring triticale was estimated. The statistical analysis showed that grain yield positively and significantly correlated with A and SPAD values throughout the growing season.

Keywords Growth stage · Leaf gas exchange · Maximum quantum yield of PSII (F_v/F_m) · N level · *Triticosecale* Wittm.

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✉ Daiva Janusauskaite
daiva.janusauskaite@lzi.lt

1 Department of Plant Nutrition and Agroecology, Lithuanian Research Centre for Agriculture and Forestry, Institute of Agriculture, Instituto 1, Akademija, Kėdainiai district LT-58344, Lithuania

2 Department of Soil and Crop Management, Lithuanian Research Centre for Agriculture and Forestry, Institute of Agriculture, Instituto 1, Akademija, Kėdainiai district LT-58344, Lithuania

Introduction

Scenarios for 2050, without considering climate change, predict that the demand for cereals in the world will increase between 42 and 59% and prices will rise between 13 and 30% (Fischer et al. 2014). One option to avoid a crisis in production of staple crops is to increase supply by increasing crop yield. Triticale is one of the many crops which possess wide application in many areas. More than 90% of crop biomass is derived from photosynthetic products. Nitrogen and water supply are very important factors governing yield (Uribelarrea et al. 2009; Olszewski et al. 2014). Nitrogen nutrition plays a decisive role in determining the process of photosynthesis of plants in agricultural environment and biomass accumulation (Sieling et al. 2016). Improvement of photosynthesis and its adaptation to environmental conditions is one of the agronomic goals (Gonzalez et al. 2010).

Leaf gas exchange can be influenced by many factors, including age, leaf architecture and position (Olszewski et al. 2014) as well as environmental factors such as air temperature, light, and nutrition (Wu et al. 2014) and water regime (Garofalo and Rinaldi 2015). The process of photosynthesis is markedly affected by climate change and varying moisture and warmth regimes (Hura et al. 2011). Photosynthetic traits significantly differ between plant genotypes (Gonzalez et al. 2010), growth stages (Janušauskaitė et al. 2013; Olszewski et al. 2014) and there may even be differences between nutrition levels (Uribelarrea et al. 2009; Feng et al. 2015).

Nitrogen level affects physiological traits of plants; however, to our knowledge, there is little information on the effect of nitrogen on the photosynthesis of spring triticale (*Triticosecale* Wittm.). Photosynthetic rate and stomatal conductance are strongly influenced by nitrogen supply level in rice (Hirasawa et al. 2010) and spring wheat (Olszewski et al. 2014). Appropriate nitrogen supply is advised to improve photosynthetic efficiency of winter wheat (Shangguan et al. 2000). A positive response of leaf gas exchange variables to nitrogen nutrition in several other crops has been widely reported (Uribelarrea et al. 2009; Li et al. 2013; Živčák et al. 2014). Deficiency in nitrogen disrupts the behaviour of photosynthetic apparatus and reduces PSII photochemical efficiency (Jiang et al. 2015; Lin et al. 2013). Under low nitrogen level, the weaker photosynthesis is often attributed to the reduction in chlorophyll content, nitrate reductase activity and Rubisco (Pal et al. 2005; Krček et al. 2008; Hirasawa et al. 2010; Li et al. 2013).

Chlorophyll is a major pigment involved in plant photosynthesis. Increases in nitrogen availability have been shown to correspond with increased leaf chlorophyll content (Schlemmer et al. 2013). The reduction of chlorophyll content may restrict photosynthetic process and, consequently, decrease of assimilate production (Cabrera-Bosquet et al. 2009). Chlorophyll fluorescence parameters are commonly used to evaluate the functionality of photosynthetic apparatus and the effects of environmental stress (Feng et al. 2015; Kalaji et al. 2016). The chlorophyll fluorescence indices can be used as general diagnostic criteria for plant nitrogen status (Feng et al. 2015). Chlorophyll fluorescence was shown to be significantly affected by nutrient supply, with increases in F_v/F_m (maximum quantum yield of PSII) in winter wheat (Li et al. 2013; Feng et al. 2015), naked oats (Lin et al. 2013) with increasing N levels. Deficiency of nitrogen reduced PSII photochemical efficiency (Kalaji et al. 2014). To our knowledge, most of the research on the effect of nitrogen supply on the leaf gas exchange and F_v/F_m of spring triticale was conducted under controlled conditions in a greenhouse as pot or hydroponic experiments. However, under field conditions this issue has not been extensively studied so far. The aims of the current field experiment were (1) to determine leaf gas exchange parameters at the main growth stages of spring triticale as affected by different N levels; (2) to compare the influence of N fertilizer form on leaf gas exchange; (3) to assess the degree of interplay among grain yield, leaf gas exchange variables and F_v/F_m. We hypothesize that N supply significantly affects the leaf gas exchange parameters of spring triticale.

Materials and methods

Site and soil description

A field experiment was conducted at the Institute of Agriculture, Lithuanian Research Centre of Agriculture and Forestry in Central Lithuania (55°23′50″N and 23°51′40″E) in 2008–2011 growing seasons. The soil of the experimental site is *Endocalcari–Endohypogleyic Cambisol (CMg-n-w-can)*. The main soil characteristics (at 0–25 cm sampling depth) of the experimental site were examined annually at the beginning of the experiment. Soil-available phosphorus and potassium were determined by ammonium lactate extraction (A-L method) (Egnér et al. 1960). The content of available P_2O_5 varied from 98 to 168 mg kg^{-1}, available K_2O from 133 to 148 mg kg^{-1}. pH_{KCl} was 5.5–6.7 (potentiometrically). The content of mineral N was 33–55 kg ha^{-1} in 0–40 cm soil layer (N–NO_3—ionometrically, N–NH_4—spectrophotometrically).

Treatments and agronomic management

A spring triticale (*Triticosecale* Wittm.) cultivar Nilex was used in the field experiments. The crop was sown at a density of 4 million viable seeds ha^{-1}. Spring barley (*Hordeum vulgare* L.) was a pre-crop. Six N levels were tested (1) no N application (N_0), (2) 90 kg N ha^{-1} (N_{90}), (3) 180 kg N ha^{-1} (N_{180}), (4) 90 + 30 kg N ha^{-1} (N_{90+30}), (5) 90 + 30 + 30 kg N ha^{-1} ($N_{90+30+30}$), (6) 90 + 30 + 30 kg N ha^{-1} ($N_{90+30+30}$). Nitrogen application time and fertilizer forms are presented in Table 1.

For all treatments, 66 kg ha^{-1} P_2O_5 [as granular monocalcium phosphate $Ca(H_2PO_4)_2H_2O$] and 130 kg ha^{-1} K_2O (as KCl) were applied pre-sowing. The experiment was laid out in a complete randomized block design with four replications. The plot size was 20.4 m^2 (2.1 m × 9.7 m).

Weed control, diseases and pest management were carried out in accordance with the crop development as required. At the beginning of steam elongation (BBCH 30), chemical weed control was performed using MCPA (1.3 l ha^{-1}, a.i. MCPA 500 g l^{-1}) + Grodyl 75 WG (0.030 kg ha^{-1}, a.i. amidosulfuron 750 g kg^{-1}) in each experimental year. The fungicides were applied at the onset

Table 1 Nitrogen fertilizer application time

Treatment no	Time and rates of N application kg ha^{-1}		
	Pre-sowing	Stem elongation (BBCH 32–33)	End of booting—beginning of heading (BBCH 49–51)
1	0	–	–
2	90 (AN[a])	–	–
3	180 (AN)	–	–
4	90 (AN)	30 (AN)	–
5	90 (AN)	30 (AN)	30 (AN)
6	90 (UAN[b])	30 (UAN)	30 (UAN)

[a] AN ammonium nitrate NH_4NO_3 (34% N)

[b] UAN urea ammonium nitrate solution $CO(NH_2)_2 \cdot nNH_4NO_3 \cdot mH_2O$ (32% N)

of foliar diseases: in 2008, 2009 and 2010, Falcon 460 EC (0.6 l ha^{-1}, a.i. tebuconazole 167 g l^{-1} + triadimenol 43 g l^{-1} + spiroxamin 250 g l^{-1}) at the end of heading (BBCH 59). The chemical insect control was performed using the following products: Karate Zeon 5 CS (0.20 l ha^{-1}, a.i. lambda-cihalotrin) at BBCH 59 in 2008 and Fastac 50 (0.2 l ha^{-1}, a.i. alfa-cipermetrin 50 g l^{-1}) at flag leaf stage (BBCH 39) in 2010.

Each year, the plots were harvested within the first 10-day period of August at complete maturity (BBCH 89) with a plot harvester "Wintersteiger Delta" (Germany). Grain yield as Mg ha^{-1} was adjusted to 15% moisture content.

Measurement of physiological parameters and SPAD

Five physiological characteristics were quantified: maximum quantum yield of PSII (F_v/F_m), photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s) and instantaneous water use efficiency (WUE). Measurements of all physiological parameters were made between 10 A.M. and 14 P.M. (local time) on clear days four times per growing season—at the beginning of stem elongation (BBCH 31), in the middle of stem elongation (BBCH 32–33), at the end of heading (BBCH 59) and from early milk of grain—to medium milk (BBCH 73–75).

The maximum quantum yield of PSII (F_v/F_m) was obtained from the emission of chlorophyll-α fluorescence, measured in vivo using a pulse modulated handheld chlorophyll fluorometer (model OS-30p; Manufacturer Opti-Sciences, Inc., Hudson, NH, USA). The maximum quantum efficiency of PSII photochemistry, F_v/F_m, was directly read after short dark adaptation on the chlorophyll fluorometer. Leaves of spring triticale were adapted to darkness for 1 min using light withholding clips. The actinic light intensity was 1000 µmol m^{-2} s^{-1}, modulation intensity two arbitrary units. F_v/F_m measurements were made on the 1st fully expanded and healthy, randomly selected leaves of 3 plants per each plot (12 plants per treatment).

Gas exchange parameters (A—photosynthetic rate, E—transpiration rate, g_s—stomatal conductance) were measured using a portable infrared gas analyser (SRS-1000) (ADC BioScientific Ltd., UK). The SRS-1000 system consists of the compact programming console and leaf chamber, which has a square (6.25 cm^2). The photosynthetic data and calculations are displayed and recorded. Measurements were done under atmospheric CO_2 and ambient light conditions. Relative air humidity (RH) during measurements differed between years. In 2008, RH was 56% and 57% at BBCH 31 and BBCH 32–33 67%, 72% at BBCH 59 and 73–75. In 2009, RH was 71–74%, in 2010—77–81% and in 2011—70–82%. The flag leaf was enclosed in the assimilation chamber and gas exchange parameters were recorded in data logger in about 2 min, when no noticeable changes in leaf respiration were registered. The measurements were made on randomly selected three plants per plot on the first fully expanded leaf from the top. The values of A and E were used to calculate the instantaneous water use efficiency (WUE = A/E).

Chlorophyll index (SPAD) was measured using a chlorophyll meter Minolta SPAD 502 (Minolta Camera Co. Ltd., Osaka, Japan). The instrument measures a difference between the absorption of light with a wavelength of 650 nm (the maximal absorption of light by chlorophyll *a* and *b*) and that of 940 nm (light absorbed by leaf tissues). Quotients of these differences are displayed in the form of SPAD units. Our measurements were made in the middle part of the fully expanded, randomly selected flag leaves of 5 plants per each plot. The mean value of four plots from the same treatments was then calculated. SPAD measurements were made from 10 A.M. until 14 P.M. (local time) on clear days four times per growing season (measured at the same time as all physiological parameters)—at BBCH 31, BBCH 32–33, BBCH 59 and BBCH 73–75.

Meteorological conditions

Rainfall differed markedly between years (Table 2). The conditions of plant growing season were described using hydrothermal coefficient (HTC) as the agrometeorological indicator, which was calculated according to the formula:

$$\mathrm{HTC} = \Sigma p/0.1\Sigma t,$$

where Σp represents sum of precipitation (mm) during the test period, when average daily air temperature is above 10 °C, and Σt denotes the sum of active temperatures (°C) during the same period.

Statistical analysis

A two-way ANOVA was used to determine year and nitrogen fertilization effects on grain yield. A three-way ANOVA was used to determine year, growth stage and nitrogen fertilization effects on gas exchange parameters. Statistical significance was evaluated at the $P \leq 0.05$ and $P \leq 0.01$ probability levels. Standard statistical procedures were used for calculating simple correlation coefficients and linear regression equations. The statistical analysis was done using software STATISTICA Base, version 6.

Results

In 2008, the total amount of rainfall between the months of April through July was 148.7 mm, or 66% of the long-term mean, and the growing period is defined as the moderate drought (HTC 0.7). The growing seasons in 2009 and 2010 matched conditions of excess irrigation (HTC 1.8). In 2009, the total rainfall reached 298.4 mm and was by 33% higher than the long-term mean. The rainfall of the growing period in 2010 totalled 359 mm and was by 44% higher, compared to the long-term average. The mean air temperature was 1.0, 0.7 and 1.8 °C above the long-term average in 2008, 2009 and 2010, respectively.

Gas exchange parameters

Photosynthetic rate (A) and transpiration rate (E), water use efficiency (WUE) and stomatal conductance (g_s) were influenced by the experimental year (factor A), growth stage (factor B) and N fertilization (factor C) (Table 3). The analysis of variance showed that year, growth stage, N rate and their interactions significantly ($P \leq 0.01$) influenced A (Table 4). N rate and growth stage explained 12.3 and 11.5% of total variability of A, respectively. Year × growth stage interaction explained the largest part (27.8%) of the total variability of A. The trial mean of A was 5.8 µmol CO_2 m^{-2} s^{-1}. The highest A value (6.9 CO_2 m^{-2} s^{-1}) was recorded in 2008. Throughout the triticale growth stages, the lowest values of A were measured at BBCH 31, then they sharply increased at BBCH 32–33 and slightly decreased during the last two measurements. On average, A value in fertilized treatments was 6.1 µmol CO_2 m^{-2} s^{-1}. The contrast of A values in fertilized (2 + 3 + 4 +5 +6) vs. non-fertilized (1) treatment was 1.8 µmol CO_2 m^{-2} s^{-1}. On average, N fertilizer application increased A values by 14–70%. Maximum A values (7.2 and 7.3 µmol CO_2 m^{-2} s^{-1}) were found in N splitting regime $N_{90} + N_{30} + N_{30}$ (treatments 5 and 6) independently of N fertilizer form (granular or liquid).

Year and growth stage significantly ($P \leq 0.01$) influenced E and explained 20.2 and 11.3% of total variability of E values, respectively. Significant effect ($P \leq 0.01$) of interactions of year × growth stage ($A \times B$) and year × growth stage × N rate ($A \times B \times C$) were also observed, while the impact of N rate and its interactions with other factors were insignificant on E (Table 3). Throughout experimental years E values ranged from 2.0 to 3.5 mmol H_2O m^{-2} s^{-1}. The highest E value (3.5 mmol H_2O m^{-2} s^{-1}) was recorded at BBCH 32–33. The contrasts of E in fertilized (2 + 3 + 4 +5 +6) vs. no fertilized (1) treatment and in ammonium nitrate (5) vs. fertilized with UAN (6) were negligible (0.2 mmol H_2O m^{-2} s^{-1}) (Table 3).

Year, growth stage, N fertilization and interaction of year × growth stage significantly influenced WUE

Table 2 Average temperatures and amounts of rainfall between the months of April through July during the experimental years 2008–2010

Year	Air temperature (°C)			Rainfall (mm)			HTC* and evaluation
	°C	Long-term average	Difference from long-term average	mm	Long-term average	% From long-term average	
2008	13.8	12.8	+1.0	148.7	223.7	66	0.7 moderate drought
2009	13.5	12.8	+0.7	298.4	224.5	133	1.8 excess humidity
2010	14.7	12.9	+1.8	352.8	226.0	144	1.8 excess humidity

The normal HTC was 1.4 for the period from April through July, when temperature was higher than 10 °C

* *HTC* hydrothermal coefficient

Table 3 Main factors effects (year, growth stage and N fertilization) on photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), water use efficiency (WUE, μmol $mmol^{-1}$), stomatal conductance (g_s, mol $m^{-2}s^{-1}$), maximum quantum efficiency of PSII (F_v/F_m) and chlorophyll index (SPAD)

Year (factor A)	Growth stage (factor B)	N fertilization (factor C)	A	E	WUE	g_s	F_v/F_m	SPAD
2008			6.9a	2.8b	2.62a	0.18c	0.609c	48.3b
2009			5.8b	3.5a	1.74c	0.46a	0.611b	47.4c
2010			4.6c	2.0c	2.82a	0.51a	0.633a	49.8a
Trial mean			5.8	2.8	2.39	0.38	0.618	48.5
	BBCH 31		4.4b	2.3b	1.93b	0.27b	0.639b	42.2c
	BBCH 32–33		7.4a	3.5a	2.36a	0.40a	0.644b	45.8c
	BBCH 59		5.9a	2.4b	3.00a	0.43a	0.585c	52.0a
	BBCH 73–75		5.4a	3.0a	2.29a	0.44a	0.604c	54.0a
		1 $^{A}N_0$	4.3b	2.6b	1.87b	0.38b	0.622b	44.6b
		2 $^{A}N_{90}$	4.9b	2.7b	2.34a	0.35a	0.620b	48.8a
		3 $^{A}N_{180}$	5.2a	2.8b	2.29a	0.33a	0.623b	49.6a
		4 $^{A}N_{90} + N_{30}$	5.9a	2.8b	2.30a	0.38a	0.615b	49.7a
		5 $^{A}N_{90} + N_{30} + N_{30}$	7.2a	3.0a	2.74a	0.42a	0.612b	49.5a
		6 $^{B}N_{90} + N_{30} + N_{30}$	7.3a	2.8b	2.83a	0.44a	0.615b	48.7a
Mean of fertilized treatments			6.1	2.8	2.50	0.38	0.617	49.3
Contrast fertilized (2 + 3 + 4 +5 +6) vs. no fertilized (1)			1.8	0.20	0.63	0	−0.005	4.7
Contrast fertilized with ammonium nitrate (5) vs. fertilized with UAN (6)			−0.1	0.20	−0.09	−0.02	−0.003	0.8

Means in the same column, for each factor, labelled with the same letter did not differ significantly at $P \leq 0.05$

[A] Fertilized with ammonium nitrate

[B] Fertilized with UAN—urea ammonium nitrate solution (N 32)

Table 4 Contribution (% of sum of squares) of year, growth stage and N fertilization and their interaction to total variance in photosynthetic rate (A), transpiration rate (E), water use efficiency (WUE), stomatal conductance (g_s), maximum quantum efficiency of PSII (F_v/F_m) and chlorophyll index (SPAD) of spring triticale

Factor	DF	A	E	WUE	g_s	F_v/F_m	SPAD
Year (A)	2	8.7**	20.2**	10.1**	34.9**	2.3**	2.8**
Growth stage (B)	3	11.5**	11.3**	6.9**	7.8**	11.7**	61.3**
N fertilization (C)	5	12.3**	0.9	4.7**	2.4**	0.3	8.6**
$A \times B$	6	27.8**	28.7**	32.7**	3.4**	41.5**	4.3**
A × C	10	2.9**	1.3	3.1	4.1**	2.2	1.1
$B \times C$	15	5.2**	2.2	1.1	9.5**	2.7	1.1
$A \times B \times C$	30	6.8**	7.8**	5.3	10.5**	2.3	3.2
Total		75.4	72.4	63.8	72.6	63.0	82.5

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

(Table 4). Interaction year × growth stage was responsible for the largest part (32.7%) of WUE total variability. Year, growth stage and N fertilization explained 10.1, 6.9 and 4.7% of the total variability of WUE, respectively. None of the interactions—year × N fertilization ($A \times C$), growth stage × N fertilization ($B \times C$), year × growth stage × N fertilization ($A \times B \times C$) were significant for WUE. On average over the experimental years, WUE was 2.39 μmol $mmol^{-1}$. The lowest WUE values were calculated in 2009 (1.74 μmol $mmol^{-1}$), the highest in 2010 (2.82 μmol $mmol^{-1}$) (Table 3). Throughout the growth stages, WUE increased from 1.93 to 3.00 μmol $mmol^{-1}$ during the period BBCH 31—BBCH 59 and decreased to 2.29 μmol $mmol^{-1}$ at BBCH 73–75. The WUE values varied depending on the N rate and increased from 1.87 to 2.83 μmol $mmol^{-1}$ in relation to the nitrogen supply from N_0 to $N_{90} + N_{30} + N_{30}$. The WUE was not influenced by N fertilizer form.

The effects of the main factors (year, growth stage, N rate) and their interactions on g_s were significant ($P \leq 0.01$) (Table 4). Year was found to be the main factor determining the differences (34.9%) of g_s between the treatments. Growth stage (factor *B*) and year × growth stage × N fertilization (*A* × *B* × *C*) were responsible for 7.8 and 10.5% of the total variability of g_s. The influence of N fertilizers was the weakest on g_s values and explained only 2.4% of the total variability of g_s. The highest g_s (0.51 mol m^{-2} s^{-1}) was recorded in 2010, and it was 2.8-fold lower (0.18 mol m^{-2} s^{-1}) in 2008 (Table 3). The trial mean of g_s was 0.38 mol m^{-2} s^{-1}. At BBCH 31, g_s values were the lowest (0.27 mol m^{-2} s^{-1}), then they increased at BBCH 32–33 to 0.40 mol m^{-2} s^{-1} and persisted almost at the same level until BBCH 73–75. The values of g_s reached 0.42–0.44 mol m^{-2} s^{-1} in $N_{90} + N_{30} + N_{30}$ (UAN) (6), but decreased to 0.38 mol m^{-2} s^{-1} in N_0 (1).

Maximum quantum yield of PSII (F_v/F_m) and chlorophyll index (SPAD)

F_v/F_m was significantly ($P \leq 0.01$) influenced by year, growth stage and their interaction (Table 4). The year × growth stage interaction (*A* × *B*) and growth stage (factor *B*) explained 41.5 and 11.7% of the total F_v/F_m variability, respectively. Throughout experimental years, F_v/F_m values ranged from 0.609 to 0.633 (Table 3). Throughout the growing stages, F_v/F_m values increased and were the highest (0.644) at BBCH 32–33, then at subsequent growth stages showed the decreasing trend. The effect of N fertilization (factor *C*) and interactions of *A* × *C* and *B* × *C* factors on F_v/F_m was insignificant. Contrast between fertilized treatments (2 + 3 + 4 +5 + 6) vs. no fertilized (1), as well as contrast between fertilized with different fertilizers forms [ammonium nitrate (5) vs. UAN (6)] was small and insignificant (−0.005 and −0.003, respectively) (Table 3). No significant effect of the interactions year × N fertilization (*A* × *C*), growth stage × N fertilization (*B* × *C*) and year × growth stage × fertilization (*A* × *B* × *C*) on F_v/F_m was evident.

The effect of main factors (*A*, *B*, *C*) and interaction of year × growth stage (*A* × *B*) on chlorophyll content, as assessed by SPAD readings, was significant ($P \leq 0.01$). The growth stage was the main factor determining the differences between treatments (61.3%). SPAD increased throughout growth stages and its values ranged from 42.2 to 54.0 at BBCH 31 and BBCH 73–75, respectively. N fertilization explained 8.6% of the total SPAD variability. SPAD increased in line with increasing N fertilizer rate. The lowest SPAD was measured in N_0 (44.6). N fertilization resulted in 10.5% higher SPAD values compared with N_0, while the highest SPAD was in N_{120}–N_{180} fertilized treatments (3–6). The effect of the interactions year × N fertilization (*A* × *C*), growth stage × N fertilization (*B* × *C*) and year × growth stage × fertilization (*A* × *B* × *C*) on SPAD was insignificant.

Grain yield

Grain yield averaged 4.32–4.89 Mg ha^{-1} throughout experimental years (Table 5). Contrast of no fertilized vs. fertilized (2 + 3 + 4 +5 +6 treatment) was on average 1.19 Mg ha^{-1} and significant at $P \leq 0.01$ level. Fertilization resulted in on average 26.6–38.9% higher grain yield compared with unfertilized (control) treatment. The highest variation of values—coefficient of variation (CV) 13.4%—was found in the unfertilized and slightly lower CV (10.0%) in all fertilized treatments. When triticale had been fertilized with ammonium nitrate at a rate $N_{90} + N_{30} + N_{30}$ (treatment 5), no significant differences were observed in comparison with fertilization at the same rate with liquid N fertilizer UAN (treatment 6). Ammonium nitrate applied at $N_{90} + N_{30} + N_{30}$ rate tended to increase the yield, compared with UAN. The analysis of variance showed that the effect of year (factor *A*), N fertilization (factor *B*) and their interaction (*A* × *B*) on grain yield of spring triticale was significant ($P \leq 0.05$ and $P \leq 0.01$).

Discussion

Climate and N availability are the most important factors in spring triticale production. Currently there is great interest in the development of novel, high-yielding, disease-resistant triticale varieties as well as more advanced cultivation systems with lower agronomic input and environmental impact that ensure high yield. The effect of N fertilizers on cereal grain yield has been reported in various studies (Uribelarrea et al. 2009; Olszewski et al. 2014). Knapowski et al. (2009) indicated that the highest grain yield was achieved by N_{120} application. Our findings suggested that N_{90} significantly increased the grain yield compared with higher N rates. Our results agree with those of Lewandowski and Kauter (2003), who established significant increases in triticale grain yield at increased N fertilizer levels. They also noted that N_{70} significantly increased triticale yield, but the increases at N_{140} were not always significant.

We found that grain yield was positively correlated with *A* values and SPAD, and the relationship was significant in most of the tested cases (Table 6). At BBCH 71–73, the correlation between *A* and grain yield was weak and insignificant. The values of *E*, g_s, WUE and F_v/F_m either did not correlate with grain yield or the correlation was found only in sporadic cases. This finding is in contrast to that of Bahar et al. (2009) who found that stomatal conductance had positive correlation with grain yield. A significant correlation was established between physiological

Table 5 Grain yield of spring triticale as affected by year and N fertilization

Year (factor *A*)	N fertilization (factor *B*)	Grain yield Mg ha^{-1}
2008		4.89b
2009		4.48c
2010		4.32c
Trial mean		4.56
	1 $^{A}N_{0}$	3.57b
	2 $^{A}N_{90}$	4.73a
	3 $^{A}N_{180}$	4.96a
	4 $^{A}N_{90} + N_{30}$	4.84a
	5 $^{A}N_{90} + N_{30} + N_{30}$	4.76a
	6 $^{B}N_{90} + N_{30} + N_{30}$ (UAN)	4.52a
Mean of fertilized treatments		4.76
Contrast fertilized (2 + 3 + 4 +5 +6) *vs.* no fertilized (1)		1.19**
Contrast fertilized with ammonium nitrate (5) vs. fertilized with UAN (6)		0.24 NS
Significance		
Year (*A*)		**
Fertilization (*B*)		**
A × *B*		*

Means in the same column, for each factor, labelled with the same letter did not differ significantly at $P \leq 0.05$

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

[A] Fertilized with ammonium nitrate

[B] Fertilized with UAN—urea ammonium nitrate solution (N 32), NS—not significant

Table 6 The correlation between grain yield and physiological indices of spring triticale

Growth stage	*A*	*E*	g_s	WUE	F_v/F_m	SPAD
BBCH 31	0.292*	0.125	−0.140	0.209	−0.179	0.248*
BBCH 32–33	0.388**	0.215	−0.061	0.289*	−0.221	0.301*
BBCH 59	0.246*	0.037	−0.382**	−0.122	0.171	0.289*
BBCH 71–73	0.125	0.166	−0.181	0.041	0.090	0.348**

A photosynthetic rate, *E* transpiration rate, g_s stomatal conductance, *WUE* water use efficiency, F_v/F_m maximum quantum efficiency of PSII

*, ** Correlation is significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

traits such as *A* and g_s and yield by Gonzalez et al. (2010). However, our findings conflict with those of Olszewski et al. (2014) who found no positive correlation between the grain yield of the studied cultivars of spring wheat and the photosynthetic rate in flag leaves, regardless of the nitrogen fertilization. Rizza et al. (2012) maintain that the relationship between WUE and yield is largely affected by the growth stage at which WUE is measured.

Increase of photosynthetic capacity and efficiency is one of the major conditions to raising yield potential of plants (Uribelarrea et al. 2009). Leaf nitrogen is also relevant to the study of photosynthetic performance because around 30% of leaf nitrogen from a C_3 sun plant is invested in CO_2 fixation (Evans 1989). Physiological responses of plants to nitrogen deficiency have been well documented (Li et al. 2013; Olszewski et al. 2014; Gioia et al. 2015). However, the N influence of this factor on triticale physiological response has received relatively little attention so far. It has been observed that *A* was influenced by N fertilizer application rate; however, higher leaf nitrogen does not always indicate higher photosynthetic capacity (Lin et al. 2013). The depression of photosynthesis under low nitrogen level is often explained as the result of the reduction in nitrate reductase activity, chlorophyll and Rubisco content (Pal et al. 2005; Krček et al. 2008; Hirasawa et al. 2010; Li et al. 2013). Cabrera-Bosquet et al. (2009) found that *A* was 24% significantly higher in high nitrogen than in low nitrogen treatments, and increase in *A* was proportional to the increase of grain yield. In the present study, N fertilization enhanced *A* values in all experimental years irrespective of the weather conditions (Fig. 1a). In moderate drought conditions in 2008 (HTC 0.7), significant increases of *A* were observed in all N fertilization levels in most of the measurements at different growth stages. In 2009 and 2010, which are defined as excess humidity year (HTC 1.8), significant increases in A values were found in the treatments (5 and 6) of the highest splitting rates ($N_{90+30+30}$), and in those applied with higher than N_{90} rates (treatments 3, 4, 5, 6), respectively. This is in agreement with most of the data reported in literature regarding N fertilizer application (Shangguan et al. 2000; Wu et al. 2014). Our findings of *A* variation during growth stages are consistent with those of Garofalo and Rinaldi (2015) and Lin et al. (2013) who found that *A* decreased with plant age. Bouranis et al. (2012) also ascertained a significant reduction of gas exchange throughout the crop growing season. We also found that different N fertilizer forms, ammonium nitrate and urea ammonium nitrate solution (UAN), in treatments 5 and 6, respectively, had the same effect on *A* values. These results are contrary to the study where it was found that supply of urea inhibited the photosynthetic activity, compared with ammonium nitrate (Nasraoui-Hajaji and Gouia 2014).

Transpiration can be regulated by root hydraulic conductance, which in turn is affected by nutrients through control of aquaporins, for example, by nitrate (Bouranis et al. 2012). Compared to the control treatment (N_0), N fertilizer application caused inconsiderable increase in *E* values (Fig. 1b), and the differences between treatments were insignificant. Our findings of *E* seem to be consistent with those of other researchers (Shangguan et al. 2000; Cabrera-Bosquet et al. 2009) who found that different nitrogen levels did not affect the *E* values. Our results confirm that the consistent patterns of *E* and *A* variation under the influence of nitrogen were comparable, which was also ascertained by other researchers (Lin et al. 2013) who found that the photosynthetic rates of functional leaves decreased in line with transpiration rates of target leaves.

In addition, we found out that *E* significantly correlated with other investigated physiological indices in most of the

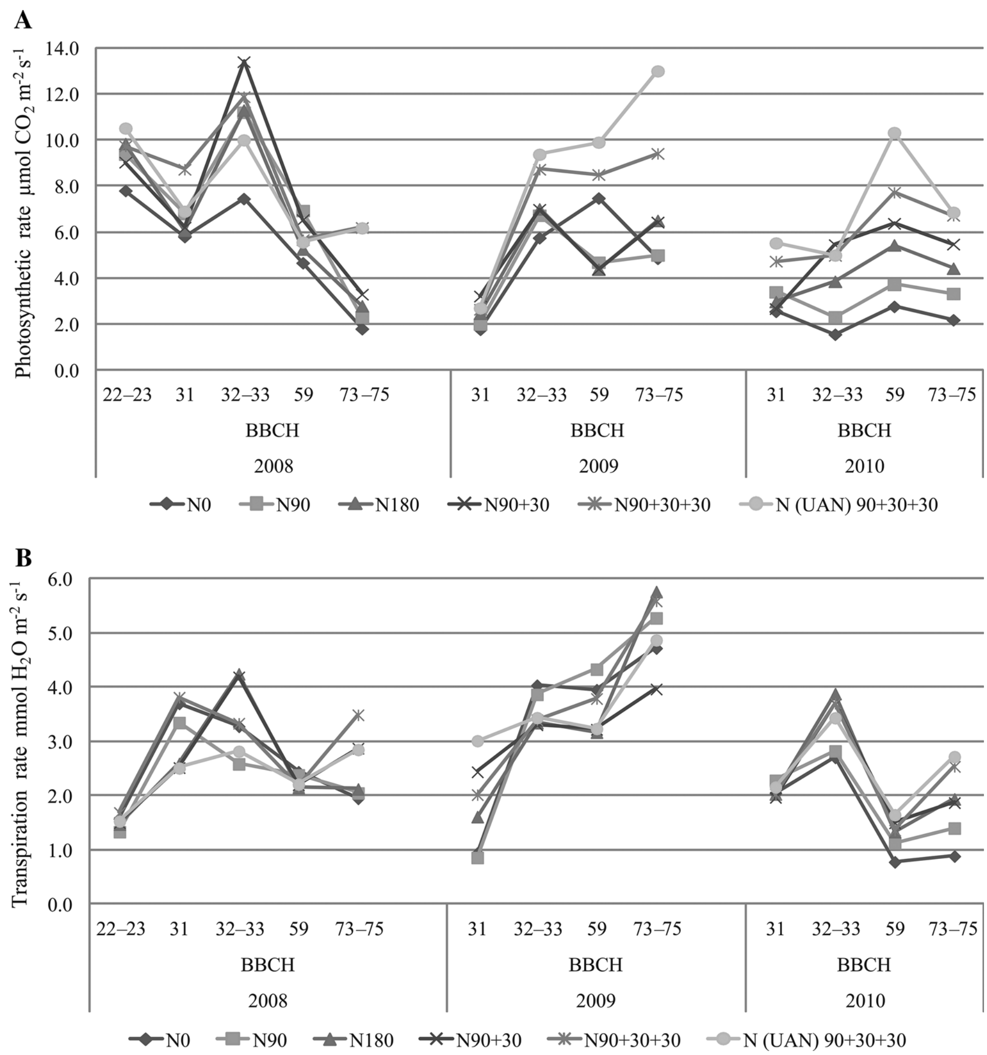

Fig. 1 Effect of N fertilizers on photosynthetic rate (**a**), transpiration rate (**b**) at different growth stages of spring triticale

tested cases, except for SPAD (Table 7). Similar data of the correlation between physiological traits are encountered in other studies (Bouranis et al. 2012; Janušauskaitė et al. 2013; Lin et al. 2013). In our study, UAN application significantly decreased *E* values, compared with N applied as ammonium nitrate (Table 3), and this finding agrees with that of Nasraoui-Hajaji and Gouia (2014) who also indicated that urea depressed *E*.

In the present study, WUE was calculated as the ratio between *A* and *E*, therefore, it is directly dependent on these two variables. Average WUE in this research differed between experimental years and ranged from 0.70 to 7.0 in 2008, from 0.95 to 3.10 in 2009, and from 0.57 to 4.91 µmol $mmol^{-1}$ in 2010 (Fig. 2a). N fertilization significantly increased WUE values compared with N_0 treatment (Table 3). This finding is in agreement with that of Shangguan et al. (2000) who showed significant influence of N fertilization on WUE and g_s under well-watered conditions. The opposite WUE trend under different N supply was reported by Loboda (2010) who found that WUE was not influenced by nitrogen doses. The lowest values of WUE were recorded at more advanced growth stages. This was determined by a decrease in *A* and *E*. We established that WUE had positive significant relation to *A* and negative significant relation to *E* values (Table 7).

Our findings indicated that g_s varied between different N levels. The application of N_{90}, N_{180} and N_{90+30} rates led to a decrease in g_s values in most of the tested cases; however, $N_{90+30+30}$ rates increased g_s, compared with N_0 (control treatment) (Fig. 2b). It is likely that these results are due to N-deficiencies resulting in major reductions of root hydraulic conductivity, which, in turn, may lead to lower stomatal conductance (Bouranis et al. 2012). Similar results were obtained by Shangguan et al. (2000) who found increases of g_s in the high-N treatment as compared to low-N treatment. In contrast, Loboda (2010) reported that g_s were not influenced by nitrogen doses irrespective of water content. As observed in our study, in 2009 and 2010, g_s was the lowest at BBCH 31, and the values significantly increased with plant aging. Bouranis et al. (2012) have also reported that g_s attains a peak value soon after leaf emergence, remains constant for a time, and declines as the leaf senesces. G_s correlated with other physiological parameters in most of the tested cases (Table 7). These findings are in agreement with those obtained by Lin et al. (2013) who found linear relationships among the physiological parameters. Bouranis et al. (2012) reported that the different relations among physiological traits to leaf WUE may be attributed to environmental effects on plant growth.

Table 7 The correlation matrix of investigated physiological indices of spring triticale

Indices	Growth stage	*E*	g_s	WUE	F_v/F_m	SPAD
A	BBCH 31	0.544**	−0.359**	0.643**	−0.594**	0.187
	BBCH 32–33	0.122	−0.299*	0.698**	−0.550**	−0.359**
	BBCH 59	0.128	0.151	0.585**	−0.174	0.116
	BBCH 71–73	0.598**	0.621**	0.280*	0.130	0.100
E	BBCH 31	–	−0.164	−0.232*	−0.523**	−0.136
	BBCH 32–33	–	0.313**	−0.487**	−0.139	0.156
	BBCH 59	–	0.19	−0.651**	−0.338**	−0.195
	BBCH 71–73	–	0.267*	−0.485**	0.266*	−0.180
g_s	BBCH 31	–	–	−0.253*	0.498**	0.155
	BBCH 32–33	–	–	−0.471**	0.291*	0.468**
	BBCH 59	–	–	0.310**	−0.328**	−0.241*
	BBCH 71–73	–	–	0.330**	0.318*	0.168
WUE	BBCH 31	–	–	–	−0.167	0.361**
	BBCH 32–33	–	–	–	−0.384**	−0.419**
	BBCH 59	–	–	–	0.147	0.220
	BBCH 71–73	–	–	–	−0.250*	0.221
F_v/F_m	BBCH 31	–	–	–	–	−0.045
	BBCH 32–33	–	–	–	–	0.457**
	BBCH 59	–	–	–	–	0.311**
	BBCH 71–73	–	–	–	–	0.252*

A photosynthetic rate, *E* transpiration rate, g_s stomatal conductance, *WUE* water use efficiency, F_v/F_m maximum quantum efficiency of PSII

*, ** Correlation is significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

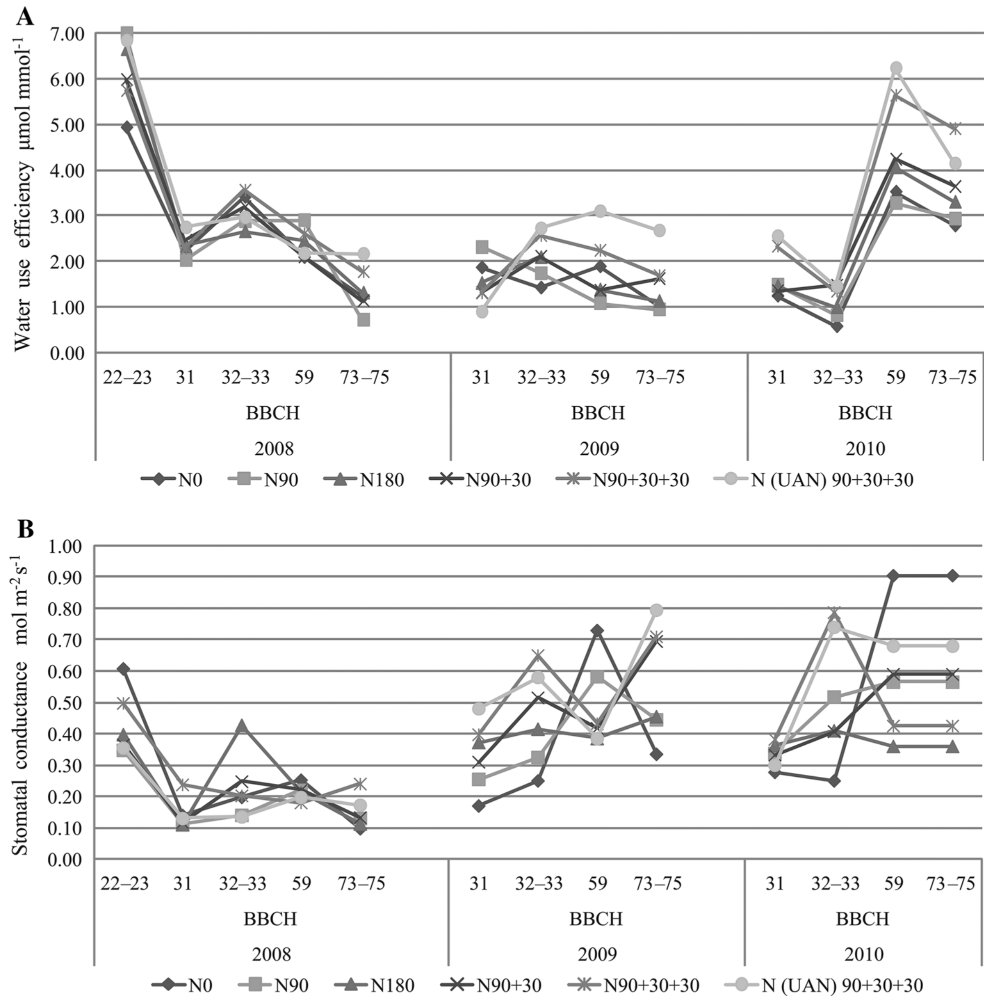

Fig. 2 Effect of N fertilizers on water use efficiency (**a**), stomatal conductance (**b**) at different growth stages of spring triticale

Previous studies have mostly focused on the use of chlorophyll fluorescence for the qualitative evaluation of plant growth and environmental stresses (Gonzalez et al. 2010; Hura et al. 2011; Sharma et al. 2015). Nutrient deficiency directly influences the photosynthetic apparatus, mainly through biosynthesis and functioning of key photosynthetic components (Živčák et al. 2014). Chlorophyll fluorescence could be considered as general diagnostic criterion for plant nitrogen status under different growing conditions (Feng et al. 2015). Nutrients have specific effects on PSII photochemistry (Kalaji et al. 2014, 2016). Currently, there is no consensus about the effects of nitrogen fertilizers on F_v/F_m. Some researchers have reported that F_v/F_m increased with increasing N application levels in naked oats (Lin et al. 2013) and in winter wheat (Shen and Li 2011). The opposite results were found by Cabrera-Bosquet et al. (2009) in durum wheat grown under different N levels, wherein the data showed a clear increase in photosynthetic gas exchange and SPAD with N fertilization, nonetheless F_v/F_m was not affected by N level. Similar results were obtained by Gioia et al. (2015) in durum wheat where the optimal nitrogen nutrition did not affect the Fv/Fm ratio compared to the treatment under nitrogen starvation conditions. Živčak et al. (2014) have reported similar data confirming that the trend of Fv/Fm in wheat leaves did not follow the trend of nitrogen supply. In agreement with above mentioned authors, our findings indicate that F_v/F_m tended to decrease in high-N level treatments, mostly at BBCH 59 and BBCH 71–73, but these differences, compared with N_0, were insignificant. It can be explained by the fact that excessive N supply can also be a stress having similar detrimental effects on F_v/F_m as N deficiency did (Zhou et al. 2006). This is consistent with the decreasing quantum yield of photosystem II that indicates impaired electron transport in the plants grown under high nitrogen supply. Additionally, we found that F_v/F_m negatively and significantly correlated with gas exchange variables and positively and significantly correlated with SPAD in most of the tested cases (Table 7).

Conclusion

This is the first study conducted under field conditions which explored the effect of different N levels on triticale gas exchange throughout the growing season. The N supply was found to have positive effect on SPAD values and keep leaves green and, therefore, more photosynthetically active. N fertilization significantly influenced A, WUE, g_s and SPAD; however, its effect on E and F_v/F_m was insignificant. At different growth stages, A values varied and reached the maximum at intensive growth period—BBCH 31–33 and BBCH 59. The highest N splitting rates had the greatest and significant effect on gas exchange variables in most of the cases.

Different N fertilizer forms, ammonium nitrate (AN) and urea ammonium nitrate (UAN), exerted relatively comparable influence on gas exchange variables, and the differences between the treatments were insignificant.

The statistical analysis revealed that spring triticale grain yield was positively and significantly correlated with A and SPAD values throughout the growing season. However, with the senescence of leaves, grain yield and A values did not correlate. The other measured variables of gas exchange (E, WUE and g_s) and F_v/F_m either did not correlate with grain yield or the correlation was found only in sporadic cases. The interdependence among gas exchange variables and F_v/F_m was significant in most of the tested cases (75%). SPAD significantly correlated only with F_v/F_m, the correlation with other gas exchange variables was rare.

These results may have practical implications for better understanding of the relationships between plant productivity and gas exchange indices in triticale. They may also come in useful for improving the productivity of spring triticale with the environmental benefits of low N input.

Author contribution statement DJ conceived and designed the study, collected and analyzed the data of the article and wrote the manuscript. DF contributed to the writing of the manuscript and statistical analyses. VF contributed with scientific advice and the correction of the manuscript. All authors have read and approved the final manuscript.

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