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Biostimulating effects of the cyanobacterium *Nostoc piscinale* on winter wheat in field experiments

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ABSTRACT

Due to global warming, a permanent rainfall deficit and higher temperatures reduce the available water in the soil, which severely influences plant water status. Current research needs to address ways to overcome these problems in order to maintain crop yields. The beneficial effects of seaweed extracts against abiotic and biotic stress factors of plant growth is well known but the use of microalgae for the same purpose is not well described. The aim of the present work was to investigate the plant biostimulating effects of the cyanobacterium *Nostoc piscinale* on the winter wheat variety "Bőség." Experiments were carried out over three years in Hungary at the Mosonmagyaróvár Faculty Farm. Freeze-dried cyanobacterium was re-suspended in water (0.3 or 1.0 g/L) and sprayed at 400 L/ha on wheat leaves at tillering or tillering and ear emergence. Root weight, relative water content (RWC), chlorophyll and proline content of leaves were measured during the vegetation period. Ear number, ear length, grain numbers in ear, thousand grain weight and yield were measured at harvest. The most economic and highest yield increase was obtained by 0.3 g/L treatment with *N. piscinale* at tillering and ear emergence. Beneficial effects included a stronger root system, elevated leaf RWC, higher proline content and increased leaf chlorophyll content, which remained high in plant leaves treated with *N. piscinale* for one or two weeks longer than in the control leaves. The high chlorophyll content extended the productive vegetation period of the treated plants. Cyanobacterium treatment increased the ear number, ear length, grain number per ear, thousand grain weight and yield of the wheat crop.

1. Introduction

Extreme weather conditions due to global warming, such as drought and higher average temperatures are great challenges for farmers not only in Hungary but in many parts of the world. Global climate change forecasts predict increasing abiotic and biotic stress effects which will reduce crop yields mainly due to drought and plant diseases (Daryanto et al., 2016). The combination of permanent rainfall deficit and high temperatures reduce of the available water in the soil, which severely influences plant water status (Anda, 2008). Current research needs to address ways to overcome these problems in order to maintain crop yields.

The beneficial effects of seaweed extracts against abiotic and biotic stress factors of plant growth were observed in 1940s, when Maxicrop, the first seaweed extract for agricultural purposes was developed and marketed (Craigie, 2011). Nowadays, there are a number of seaweed preparations mostly made from brown algae and used as plant biostimulants (Khan et al., 2009; van Staden et al., 2016). Plants treated with seaweed extracts show increased root and shoot growth, enhanced nutrient uptake, improved flowering and grain filling, higher chlorophyll content, and longer photosynthetically active vegetation periods, consequently producing higher yield (Crouch and van Staden, 1994; Khan et al., 2009; Metting et al., 1990). Treated plants are more tolerant against insects and pathogens, as well as against some abiotic stress effects such as drought and freezing (Craigie, 2011; Crouch and van Staden, 1994; Khan et al., 2009; Metting et al., 1990).

Seaweed extracts are used for soil or leaf treatment in low concentrations, therefore the favorable effects cannot be explained by the macro- and micro-elements in the extract (Craigie, 2011; Crouch and van Staden, 1994). It was established that at low concentrations plant hormones derived from the seaweed are responsible for the induction of the beneficial physiological responses (Craigie, 2011; Crouch and van Staden, 1994; Khan et al., 2009). Cyanobacteria and eukaryotic microalgae are also able to produce plant hormones (Ördög and Pulz, 1996; Ördög et al., 2013; Stirk et al., 2013) and therefore may also demonstrate plant biostimulating effects.

Abbreviations: DW, dry weight; E, ear emergence; FW, fresh weight; MACC, Mosonmagyaróvár Algal Culture Collection; N, *Nostoc* treatment; RWC, relative water content; SW, saturated weight; T, tillering.

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Microalgae may be used in the agriculture as organic fertilizers, soil conditioners, biopesticides and plant biostimulants (Stirk et al., 2004). Pepper and sunflower plants treated with the cyanobacterium *Nostoc entophytum* (MACC-612) and the green alga *Tetracystis* sp. (MACC-430) could survive a longer period without irrigation than control plants (Bajzátné Lisz, 2013; Pőthe et al., 2014). The aim of the present work was to investigate the plant biostimulanting effects of the cyanobacterium MACC-612 *Nostoc piscinale* (former *N. entophytum*) on a winter wheat variety grown in field conditions.

2. Material and methods

2.1. Experimental design

The experimental plant was the winter wheat variety *Triticum aestivum* cv. "Bőség" (Virágmag Kft, Hungary). Experiments were carried out over three years at the Mosonmagyaróvár Faculty Farm (47°52′N; 17°16′E) in 2015/16, 2016/17 and 2017/18. There were 20 plots with five treatments and four replications in a random block layout. The plot size was 10 m^2 (0.96 × 10 m) with 12 cm row spacing and 4.5 million plant density/ha. Soil type and characteristics of the experimental field are summarized in Table 1. Sowing, plant treatment and harvest times are summarized in Table 2.

Biomass of the cyanobacterium strain MACC-612 *Nostoc piscinale* obtained from the Mosonmagyaróvár Algal Culture Collection (MACC) was produced in laboratory culture units previously described (Ördög, 1982). The cyanobacterium strain was inoculated from agar–agar stock cultures into Tamiya nutrient solution (Kuznjecov and Vladimirova, 1964). Following a 7 day incubation period, the cultures were reinoculated into 4 flasks containing 250 mL Tamiya nutrient solution with a starting concentration of 10 mg/L algal dry weight (DW). The cultures were grown at 25 ± 2 °C, in a 14:10 light:dark cycle and 130 μmol photons/m^2/s light intensity illuminated from below. The cultures were aerated with 20 L/h (= 1.33 L air/L nutrient medium per min) 1.5% CO_2-enriched sterile humidified air during the light period. After 7 days, the four culture suspensions were combined, the density of the suspension measured and used for inoculation of 48 flasks to give a starting concentration of 10 mg/L DW. The cultures were grown in the conditions described above for 6 days, then centrifuged for 15 min at room temperature with 2150*g* (Sigma 6 K15). The biomass was freeze-dried (Christ Gamma 1–15) and stored at −18 °C. Biomass samples were resuspended in distilled water and sonicated 3 min (VirTis, VirSonic 600 Ultrasonic Cell Disruptor) just prior to plant treatments.

The plants were treated either with tap water (control) or with the cyanobacterium in a concentration of 0.3 or 1.0 g/L DW. These concentrations were selected based on the results obtained with sunflower (Pőthe et al., 2018) and winter rape (Tóth et al., 2016). The suspension was applied once at tillering (T; phenological phase BBCH-21) or twice at T and ear emergence (E; phenological phase BBCH-49). The treatments abbreviations include the cyanobacterium (N), concentration (0.3 or 1.0 g/L) and treatment time (T and T,E). The cyanobacterium suspension was sprayed with 400 L/ha water to the leaves of the plants with a manual sprayer. For better adhesion of the cyanobacterium to the leaf surface, Trend 90, a non-ionic wetting agent was added to the spray. The control plot was irrigated with tap water containing only the wetting agent. Plants of each plot were separately harvested with a combine harvester.

Table 1
Soil characteristics of the experimental field at the Mosonmagyaróvár Faculty Farm in the experimental years.

Soil parameters	2015/16	2016/17	2017/18
Soil type	Danube alluvial soil		
Crop grown previously	corn		
Humus content (m/m%)	3.3	3.1	3.2
Gold crown value	39		
Liquid limit (K_A)	53	51	52
pH KCl	7.2	7.1	7.2
Tilth (cm)	130		
Salt (%)	0.0	0.0	0.0
$CaCO_3$ (m/m%)	21.1	21.0	21.4
Al-P_2O_5 (mg/kg)	186	183	189
Al-K_2O (mg/kg)	199	195	200
NO_2-NO_3-N (mg/kg)	11.8	11.9	12.8

Table 2
Sowing and harvest time and time of first- and second treatments with *Nostoc piscinale* MACC-612 at the Mosonmagyaróvár Faculty Farm in the experimental years.

		Dates		
		2015/16	2016/17	2017/18
Sowing time	Phenological phase	27.10.2015	27.10.2016	06.11.2017
First treatment	BBCH-21; tillering	12.03.2016	01.04.2017	14.04.2018
Second treatment	BBCH-49; ear emergence	21.05.2016	20.05.2017	16.05.2018
Harvest time		21.07.2016	05.07.2017	02.07.2018

2.2. Root dry weight

The root DW of the plants collected from the middle three rows of each plot was determined 2 days before and 10 days after the first treatment. It was not possible to measure the root DW after the second treatment as the root system was too well developed and thus not possible to dig out. Ten plants were removed (30 × 30 cm field) from each plot. The roots were cleaned from soil particles and dried at 106 °C to a constant weight for 24 h, cooled down and measured with an analytical balance.

2.3. Relative water content of leaves

The relative water content (RWC; %) of the flag leaves was measured weekly (3 plants/plot) by the method of Cabrera-Bosquet et al. (2009), starting one week after the first treatment. The RWC shows the proportion of the actual water content to the water content of the saturated leaf. The weight of the freshly cut flag leaves (fresh weight, FW) was measured, immersed/dipped in water for 24 h (saturated weight, SW) and then dried in oven (60 °C) (DW) for 24 h. The relative water content was calculated using the following formula:

RWC % = (FW-DW)/(SW-DW) × 100.

The average RWC of the 3 plants/plot was used in the statistical analysis.

2.4. Chlorophyll content

The chlorophyll concentration of the flag leaves was determined with the portable device: SPAD 502 Plus Chlorophyll Meter. The chlorophyll measurement of 5 plants/plot commenced one week after the first treatment and was carried out for 5 days a week, always around 4 pm. The chlorophyll content was expressed in SPAD unit. The average SPAD value of the 5 plants/plot was used in the statistical analysis.

2.5. Proline content

The proline content of the flag leaves (0.3 g FW) was quantified using the method of Bates et al. (1973). Measurements commenced one week after the first treatment and was carried out once a week for the duration of the field trial. The proline contents of 4 plants/plot were measured and their average was used in the statistical analysis. The proline concentration (μg/mL) were determined from the standard curve and the proline content of the biomass was calculated on a fresh weight basis as follows:

[(μg proline/mL × 3 mL toluene)/115.5 μg/μmol]/[0.3 g sample/5] = μmol proline/g FW weight.

Table 3
Distribution of monthly precipitation and average monthly temperature in the winter wheat growing seasons of the experimental years at the Mosonmagyaróvár Faculty Farm compared with the average values of the past 50 years (https://www.met.hu/ 27.03.2019). Values in bold are higher and values in italics are lower than the 50 years average.

Month	Experimental years						50 year average (1966–2016)	
	2015/16		2016/17		2017/18			
	mm	°C	mm	°C	mm	°C	mm	°C
October	**91**	*9.8*	**63**	*9.7*	**56**	**12.2**	41	10.4
November	*22*	**6.9**	**62**	5.0	*44*	**6.0**	47	5.0
December	*11*	**2.7**	*8*	*0.4*	*37*	**2.5**	39	1.1
January	**45**	**−0.6**	*15*	*−4.6*	*30*	**3.2**	36	−1.2
February	**82**	**5.8**	*24*	**2.7**	*31*	*−0.5*	33	0.8
March	*12*	**6.4**	*26*	**8.9**	*34*	*3.8*	35	5.2
April	*15*	**11.3**	*35*	*10.4*	*24*	**15.5**	39	10.5
May	**75**	**15.7**	*27*	**16.5**	**64**	**18.9**	57	15.4
June	*58*	**20.2**	*42*	**21.9**	**107**	**21.2**	64	18.7
July	**120**	**22.1**	*49*	**22.1**	*58*	**21.9**	65	20.4
Total/Average	531.0	10.0	351.0	9.3	485.0	10.5	456.0	8.6

2.6. Plant parameters

The following parameters of 30 plants harvested from each plot were measured – ear number/m^2, grain number/ear, ear length and thousand grain weight. The yield (t/ha) was calculated from the yield harvested from each plot. The grain quality was characterized by the protein (%) and gluten (%) content and by the Zeleny number (mL), all measured with a Foss Infratec 1241 grain analyzer.

2.7. Statistical analysis

The average values of 3–30 plants/plot were used to the statistical analysis of the experiments carried out in 4 plots/treatment. The results were analyzed using Dell Statistica 13.2 software and the Microsoft Excel^R 2010 spreadsheet software. The Student t-test was used to calculate the standard deviations of the 4 replicates (plots) within each treatment. Significant differences between the control and each treatment were calculated using the Duncan test.

3. Results

3.1. Precipitation and temperature

The precipitation during the experimental period in 2015/16 and in 2017/18 was 16% and 6% higher than the 50 years average, respectively. Decreased precipitation (23%) was measured in 2016/17. The distribution of precipitation was uneven in all experimental years, but was favorable for winter wheat except in the spring months of 2017 (Table 3).

The average temperature during the experimental period was higher in all experimental years than the 50 years average (Table 3). The average temperature in Oct and Nov was higher in 2015 (8.3 °C) and in 2017 (9.1 °C) and lower in 2016 (7.3 °C) than the 50 years average (7.7 °C).

3.2. Root dry weight

Ten days after the first treatment, all cyanobacterium treated plants developed a significantly stronger root system compared to the control plants (Table 4).

3.3. Relative water content of leaves

Each year, the maximum RWC of the control leaves (88%) decreased from June onwards to a low of 20–25%. In the treated plants, the RWC of the flag leaves was always higher than in the control plants except the treatment N 0.3 g/L (T) from May 25, 2016 (Fig. 1A) and from May 23, 2017 (Fig. 1B). The highest average RWC were obtained with the N 1.0 g/L (T,E) treatment (Fig. 1).

3.4. Chlorophyll content of leaves

All treated plants revealed higher leaf chlorophyll content presented as SPAD-value than the control plants in all experimental years. The control values were around 45 SPAD unit in the last days of May and then started to decrease thereafter. A similar decrease was measured in the N 0.3 g/L (T) and N 1.0 g/L (T and T,E) treatments while this decrease was delayed in the N 0.3 g/L (T,E) treatment in the first two years (Fig. 2A and B). In the third year, the chlorophyll content started to decrease 2 days earlier and the chlorophyll content of the microalgae treated plants also decreased more rapidly (Fig. 2C).

3.5. Proline content of leaves

In all experimental years, the proline concentrations of all cyanobacterium treated plants was higher than the control, with some exceptions in 2016/17. The highest values were measured in the N 1.0 g/L (T and T,E) plants and lower values in the N 0.3 g/L (T and T,E) plants one week after the first treatment. Thereafter, the proline content decreased continuously but remained higher than the control at the end of the measuring period (Fig. 3).

3.6. Plant parameters

In all experimental years, the ear numbers (m^2) of cyanobacterium treated plants were higher than the control. In the experimental years 2015/16 and 2017/18, the plants treated twice, while in 2016/17 plants treated only once had a significantly higher ear number than the control. The ears were longer (cm) in all treatments compared to the control in 2015/16 and 2017/18. In 2016/17, the N 0.3 g/L (T) plants and the N 1.0 g/L (T and T,E) were higher than the control (Table 5).

Table 4
Root DW of the winter wheat variety "Bőség" collected from all control and cyanobacterium treated (N) plots two days before (1) and 10 days after (2) the first treatment in the experimental years. Results are presented as mean ± SD where n = 4. Different letters indicate significant differences and values in bold are significantly higher than the control ($P < .05$).

Treatments	Experimental years					
	Root DW/plant (g)					
	2015/16		2016/17		2017/18	
	1	2	1	2	1	2
Control	0.48 ± 0.1^b	0.55 ± 0.1^c	0.49 ± 0.1^a	$0.52 \pm 0.1_d$	0.75 ± 0.0^b	0.80 ± 0.0^c
N, 0.3 g/L (T)	0.49 ± 0.0^a	**0.94 ± 0.0^b**	0.42 ± 0.1^d	**0.97 ± 0.1^b**	0.75 ± 0.1^b	**1.00 ± 0.1^b**
N, 1.0 g/L (T)	0.49 ± 0.0^a	**0.94 ± 0.0^b**	0.49 ± 0.0^a	**0.94 ± 0.0^b**	0.73 ± 0.0^c	**1.12 ± 0.0^a**
N, 0.3 g/L (T,E)	0.48 ± 0.1^b	**1.05 ± 0.1^a**	0.46 ± 0.1^c	**1.05 ± 0.1^a**	0.76 ± 0.1^a	**1.13 ± 0.1^a**
N, 1.0 g/L (T,E)	0.46 ± 0.1^c	**1.02 ± 0.1^a**	0.48 ± 0.0^b	**0.88 ± 0.0^c**	0.70 ± 0.0^d	**1.15 ± 0.0^a**

The grains per ear and the thousand grain weight of treated plants were significantly higher than the control in all years with the highest values in the N 0.3 g/L (T and T,E) plants (Table 6).

The yields of all treated plants were significantly higher than the control in all experimental years. The highest average yield increase was in the plots treated twice (T, E) with 0.3 and 1.0 g/L (Table 7).

The average protein-, gluten- and Zeleny values of the grains were generally not influenced by the treatments (data not shown) except in some cases: namely, the protein content in 2017/18 (14.0%), the gluten content in 2015/16 (25.0%), and the Zeleny value in 2015/16 and in 2017/18 (44.4 and 55.8 mL) increased in plants treated twice with *N. piscinale* (1 g/L).

4. Discussion

A bigger root system increases the drought tolerance of plants under rapidly changing environmental conditions (Sheng and Hunt, 1991). The root system of some winter wheat varieties penetrate faster into the soil in early spring than other wheat varieties (Danilchuck, 1972). In the present experiment, the spring treatment of N 0.3 g/L (T) significantly increased the average root DW of the winter wheat compared to the control. Similar results were obtained with the wheat variety Inia when treated with the seaweed concentrate Kelpak five weeks after planting with the root DW significantly higher than in the control plants (Nelson and Van Staden, 1986). When Kelpak was applied on spring wheat in different doses (2 and 1.5 L/ha) and developmental stages

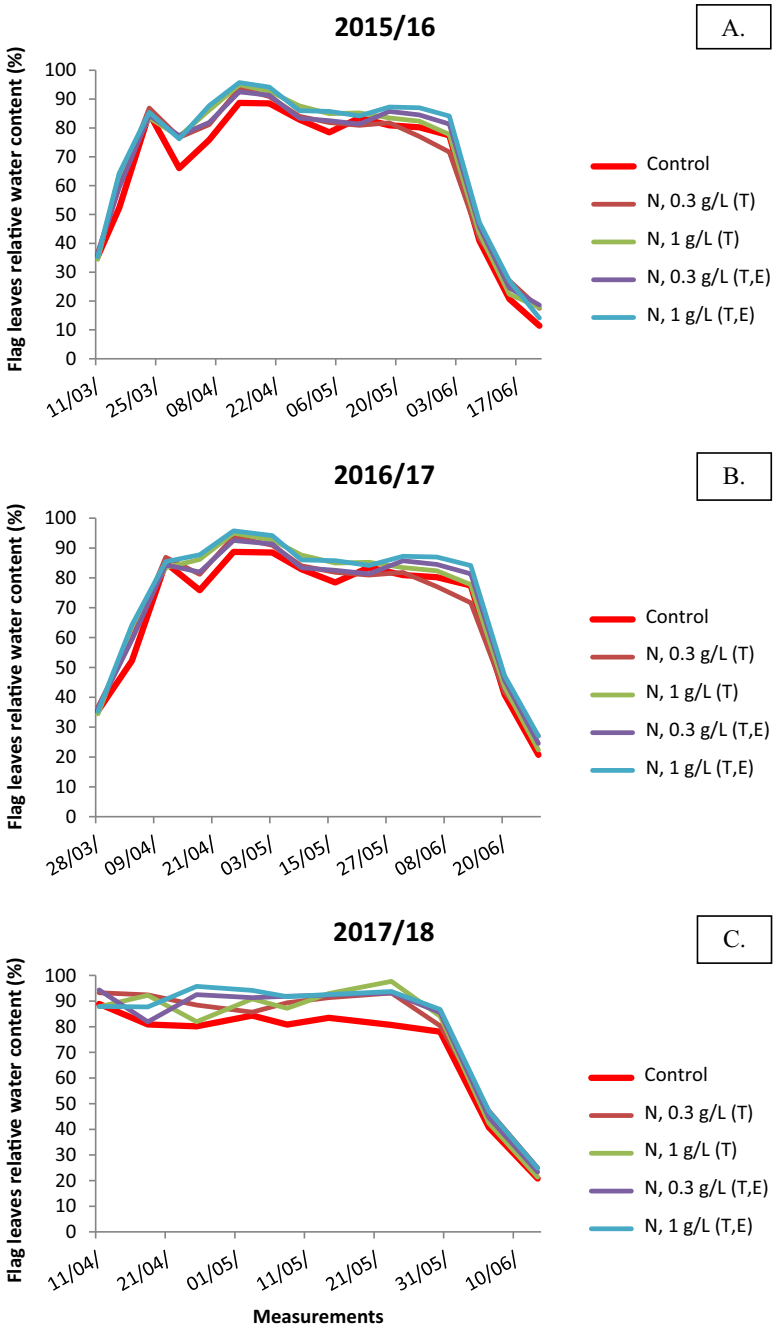

Fig. 1. Flag leaves relative water content (%) of the winter wheat variety "Bőség" measured weekly during the vegetation period in field experiments conducted in (A) 2015/16, (B) 2016/17 and (C) 2017/18. The measurements began a week after the first treatment with 0.3 or 1.0 g/L *N. piscinale* at tillering (n = 4).

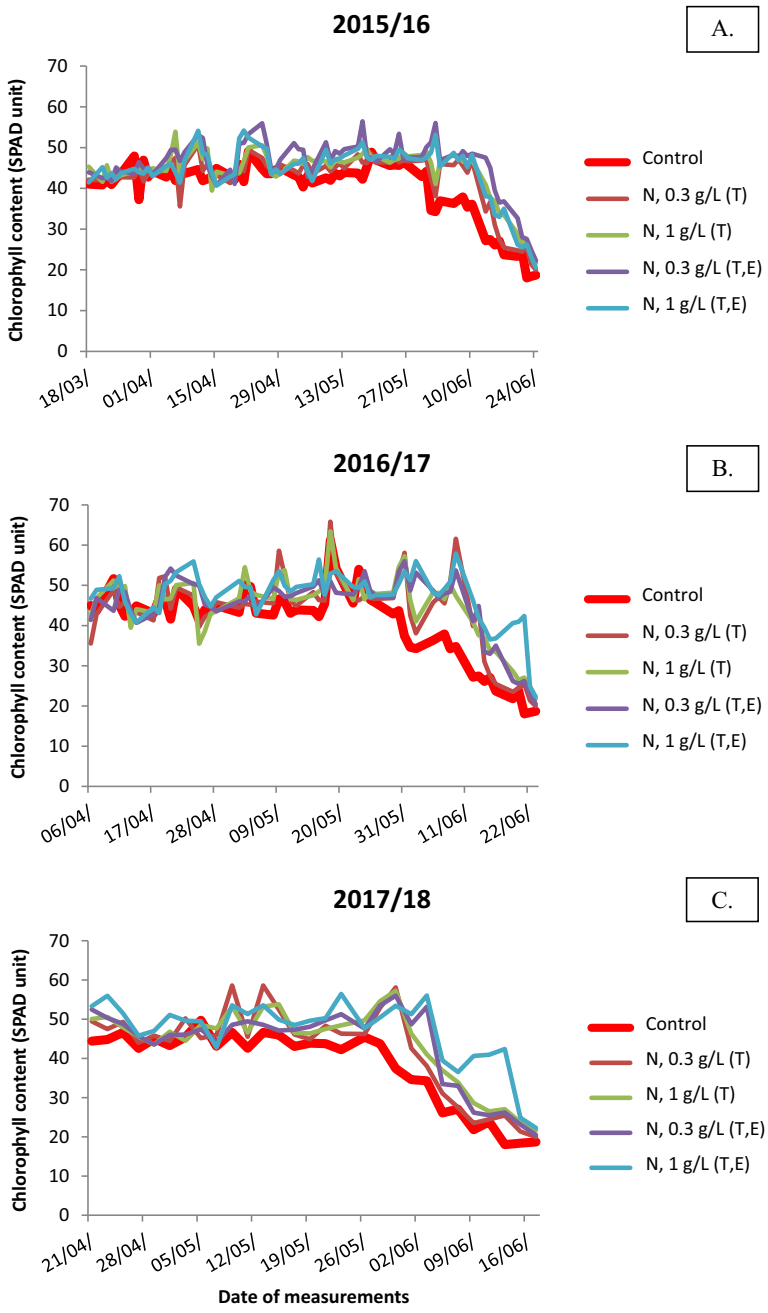

Fig. 2. Flag leaves chlorophyll content in SPAD unit of the winter wheat variety "Bőség" measured 5 days a week at 4 pm during the vegetation period in field experiments conducted in (A) 2015/16, (B) 2016/17 and (C) 2017/18. The measurements began a week after the first treatment with 0.3 or 1.0 g/L *N. piscinale* at tillering (n = 4).

(tillering and steam elongation) under field conditions, root DW in all treatments were significantly higher than the control (Szczepanek et al., 2018).

The leaf RWC is a good parameter to indicate stress by showing the proportion of the actual and saturated water content of the leaf (Alizade, 2002). With a decrease in RWC, the osmolality of the sorghum leaf increased and the slow development of water deficiency resulted not only in osmotic adaptation, but also in reduced elasticity of leaf tissue (Jones and Turner, 1978). In general, the RWC is higher in drought-resistant plants than in sensitive plants (Saeidi et al., 2015). In the present study, the RWC values were above 80% in the control plant leaves of the "Bőség" variety in April and May of all experimental years which suggests the drought tolerance of this variety. The RWC values were further increased by the cyanobacterium treatments with the highest average RWC values measured in the N 1.0 g/L (T,E) treated plant. The average RWC values of the other treatments in decreasing order were: N 0.3 g/L (T,E) > N 1.0 g/L (T) > N 0.3 g/L (T). When three drought-sensitive and three drought-resistant wheat genotypes were grown under drought stress conditions, the drought-resistant varieties (Varinac, Sardari, Kavir,) had a higher RWC content (72.2%, 74.4% and 79.9%) than the drought-sensitive ones (Ghods, Tajan, Marvdasht – 59.3%, 64.3% and 73.2%; Arjenaki et al., 2012). A high (78.8%) relative water content was measured in Marvdasht wheat genotype under drought stress and also in ideal water supply conditions (Saeidi et al., 2015). The highest RWC-values were measured in drought tolerant wheat genotypes under drought stress conditions (Schonfeld et al., 1988).

Wheat plants do not take up nitrogen from the soil after flowering. The nitrogen is transported from the leaves to the seed, which is derived from the RUBISCO enzyme (Taiz et al., 2015). The decreased RUBISCO

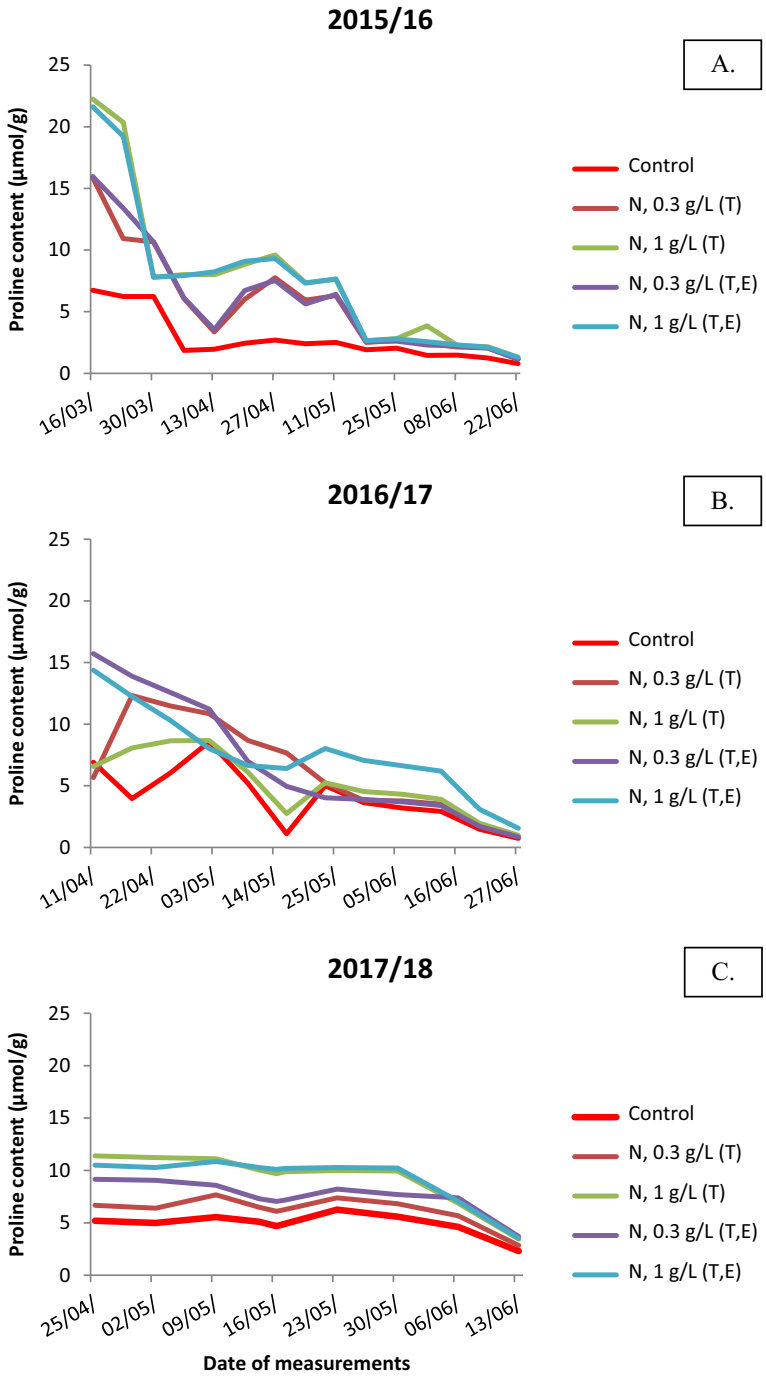

Fig. 3. Flag leaves proline content (µmol/g) of the winter wheat variety "Bőség" measured weekly during the vegetation period in field experiments conducted in (A) 2015/16, (B) 2016/17 and (C) 2017/18. The measurements began a week after the first treatment with 0.3 or 1.0 g/L *N. piscinale* at tillering (n = 4).

Table 5
Ear number and ear length of winter wheat variety "Bőség" treated with *N. piscinale* at tillering (T) or at tillering and ear emergence (T,E) in the experimental years. Results are presented as mean ± SD where n = 4. Different letters indicate significant differences and values in bold are significantly higher than the control ($P < .05$).

Treatments	Ear number (m^2)			Ear length (cm)		
	2015/16	2016/17	2017/18	2015/16	2016/17	2017/18
Control	443.8 ± 8.8^{e}	400.0 ± 47.6^{e}	428.1 ± 8.1^{e}	6.3 ± 0.3^{c}	6.5 ± 0.1^{b}	6.2 ± 0.2^{c}
N, 0.3 g/L (T)	**479.6** ± 10.7^{d}	**507.8** ± 72.8^{d}	**464.0** ± 7.9^{d}	6.8 ± 0.3^{b,c}	6.8 ± 0.3^{a,b}	**6.6** ± 0.3^{a,b}
N, 1.0 g/L (T)	**498.4** ± 20.7^{c}	**504.6** ± 29.0^{a,b}	**489.0** ± 25.2^{c}	**7.5** ± 0.2^{a}	6.6 ± 0.2^{b}	6.5 ± 0.2^{b,c}
N, 0.3 g/L (T,E)	**528.1** ± 8.1^{a}	**476.6** ± 76.8^{c}	**520.3** ± 18.7^{a}	**7.1** ± 0.3^{a,b}	6.5 ± 0.1^{b}	6.5 ± 0.1^{b,c}
N, 1.0 g/L (T,E)	**521.8** ± 8.1^{b}	**476.5** ± 53.0^{a}	**520.3** ± 8.1^{b}	**7.2** ± 0.2^{a,b}	**6.9** ± 0.2^{a}	**6.8** ± 0.2^{a}

Table 6
Grain number and grain weight winter wheat variety "Bőség" treated with *N. piscinale* at tillering (T) or at tillering and ear emergence (T,E) in the experimental years. Results are presented as mean ± SD where n = 4. Different letters indicate significant differences and values in bold are significantly higher than the control (P < .05).

Treatments	Grains per ear (pc)			Thousand grain weight (g)		
	2015/16	2016/17	2017/18	2015/16	2016/17	2017/18
Control	29.3 ± 1.7^{d}	36.0 ± 4.7^{a}	40.0 ± 0.8^{e}	32.0 ± 1.3^{c}	38.7 ± 0.8^{c}	38.6 ± 1.8^{c}
N, 0.3 gL (T)	**36.0** ± 1.4^{c}	39.6 ± 1.2^{a}	**46.8** ± 1.5^{a,b}	**39.5** ± 0.8^{b}	**41.7** ± 0.7^{a}	41.0 ± 2.6^{a,b,c}
N, 1.0 g/L (T)	**37.7** ± 2.2^{b}	38.0 ± 1.8^{a}	**43.8** ± 1.3^{d}	**39.8** ± 1.2^{b}	39.7 ± 1.5^{c}	**42.6** ± 1.0^{a,b}
N, 0.3 g/L (T,E)	**41.0** ± 1.8^{a}	40.0 ± 2.7^{a}	**48.0** ± 0.4^{a}	**40.1** ± 1.5^{a,b}	39.6 ± 0.8^{c}	**42.8** ± 0.8^{a}
N, 1.0 g/L (T,E)	**41.8** ± 1.6^{a}	37.8 ± 1.7^{a}	**45.8** ± 1.0^{b,c}	**41.7** ± 0.8^{a}	39.8 ± 1.7^{b,c}	39.8 ± 1.4^{c}

content of the leaves is accompanied by lower CO_2 fixation and decreased chlorophyll and RWC of the leaves. The reason for the decreased chlorophyll content is that drought or heat stress promotes the production of reactive oxygen forms, such as O_2 and H_2O_2, which can lead to lipid peroxidation and consequently chlorophyll decomposition (Foyer et al., 1994; Lessani and Mojtahedi, 2002). All wheat plants treated with *N. piscinale* had a higher leaf chlorophyll content than the control plants in all experimental years. The chlorophyll content of the control plants started to decrease at the beginning of June while in cyanobacterium treated plants, the decrease was delayed up to two weeks. Thus the photosynthetically active vegetation period was longer in the treated plants, which could be linked to the significant increase in yield (Table 7). Similarly, chlorophyll can be protected by seaweed extracts. Kelpak treatment resulted in 10% higher chlorophyll content compared to control plants (Sosnowski et al., 2013). The three drought-resistant wheat varieties had a higher chlorophyll content than the three sensitive varieties (Arjenaki et al., 2012).

Accumulation of osmolites such as proline in the cytoplasm promotes "osmolytic adjustment" of the plants by reducing internal osmotic potential and contributes to plant stress tolerance (Delauney and Verma, 1993; Chen and Murata, 2002). Plants in drought stress conditions maintain the turgor of cells by synthesis of osmolites to ensure the continuous water uptake (Maggio et al., 2002). There is a positive correlation between proline concentration and membrane integrity of wheat leaves. Proline stabilizes membrane phospholipids that help the plants to overcome drought (Mujtaba et al., 2007; Maria et al., 2008). The proline concentrations of all plants treated with *N. piscinale* in the present experiment were higher than in the control plants after the first microalgae treatment. The highest average values were measured in the N 1.0 g/L (T and T,E) plants and lowest in the control plants. The differences in the proline content (and in the leaf RWC) cannot be explained by the total precipitation and soil water contents, which were quite similar to each other in the three experimental years. Similarly, proline was lower in wheat leaf tissue grown under optimum conditions compared to those grown in water stressed conditions (Mwadzingeni et al., 2016).

The wheat yield depends on several abiotic (precipitation, temperature, soil water content) and biotic factors (pathogens, insects, soil microbes), but can also be influenced by specific plant treatments such as fertilizers, pesticides and seaweed extracts (van Staden et al., 1995).

Table 7
Yield of winter wheat variety "Bőség" treated with *N. piscinale* at tillering (T) or at tillering and ear emergence (T,E) in the experimental years. Results are presented as mean ± SD where n = 4. Different letters indicate significant differences and values in bold are significantly higher than the control (P < .05).

Treatments	Yield (kg/ha)		
	2015/16	2016/17	2017/18
Control	6041.9 ± 841.^{8e}	3253.0 ± 185.9^{d}	4913.4 ± 594.2^{e}
N, 0.3 g/L (T)	**7702.3** ± 698.4^{c}	**3635.9** ± 300.4^{c}	**5899.3** ± 337.0^{d}
N, 1.0 g/L (T)	**7684.1** ± 519.5^{d}	**4018.0** ± 414.2^{b}	**6024.1** ± 321.9^{c}
N, 0.3 g/L (T,E)	**8362.3** ± 496.0^{a}	**4203.3** ± 614.8^{a}	**6078.4** ± 246.7^{d}
N, 1.0 g/L (T,E)	**7827.8** ± 1080.1^{b}	**4191.0** ± 818.7^{a}	**6189.8** ± 833.6^{a}

In the experimental years 2015/16 and 2017/18, the plants treated twice with *N. piscinale* had significantly more ear numbers than the control and the ears were longer in all treatments. The grains per ear and the thousand grain weight of microalgae treated plants were significantly higher than the control in all years with the best yields obtained with the N 0.3 g/L (T and T,E) treatment. Similarly, the cockfoot (*Elymus repens*) variety Amila treated with the seaweed extract Kelpak had a higher yield (Sosnowski et al., 2013). Wheat treatment with Kelpak (1.5 L/ha) increased grain number per ear, thousand grain weight and yield (4947 kg/ ha) compared to the control (Szczepanek et al., 2018). However, the Kelpak in 2 L/ha had no effect on the wheat grain number per ear (Matysiak et al., 2012).

In conclusion, the highest average yield increase was in the plots treated twice (T, E) with 0.3 and 1.0 g/L *N. piscinale*. The most economic and highest yield increase was obtained by 0.3 g/L treatment with *N. piscinale* applie twice at tillering and ear emergence. The beneficial effect started with a stronger root system, followed with increased leaf chlorophyll content, which remained high in plant leaves treated with *N. piscinale* for one (in 2017/18) or two weeks (2015/16 and 2016/17) longer than in the control leaves. The high chlorophyll content increased the productive vegetation period of the microalgae treated winter wheat plants. There was also elevated leaf RWC and higher proline content in the treated plants. The cyanobacterium treatments increased the ear number, ear length, grain per ear, thousand grain weight and the yield of the winter wheat variety "Bőség."

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