



## Plant biostimulating effects of the cyanobacterium *Nostoc piscinale* on maize (*Zea mays* L.) in field experiments



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### ABSTRACT

Biostimulants, when applied to plants in small amounts, increase crop yield and plant tolerance to abiotic and biotic stress. They play an important role in the development of new environmentally sustainable technologies. The aim of the current experiment was to investigate the potential of a cyanobacterium strain (*Nostoc piscinale*) to improve the growth, grain yield and stress tolerance of maize (*Zea mays* SY Zephir hybrid). Field trials were established at two sites. Freeze-dried biomass of *N. piscinale* resuspended in tap water (1g/L DW) was applied as a single foliar treatment (400 L/ha) at the V6-V7 phenological stage. Number of leaves, chlorophyll content, relative water content (RWC%) and free proline content were measured weekly. Grain yield, yield components and grain protein content were measured at harvest. *N. piscinale* treated maize had significantly earlier development in the vegetative growth stages with a higher number of leaves. Chlorophyll content (SPAD value) was significantly higher in the treated plants during the reproductive stages. There was little difference in the RWC and proline content compared to control plants. Faster vegetative growth and higher chlorophyll content in the cyanobacterium treated plants meant great photosynthetic light absorption over a longer period of time, resulting in significantly higher grain yield (6.5% and 11.5% at the two production sites) and increased grain protein content. Grain yield was significantly influenced by cob length and thousand grain weight. In conclusion, it was proved in field trials conducted in two different regions in Hungary that a single foliar application of a cyanobacterium-based biostimulant can contribute to crop production in a sustainable and environmentally friendly manner.

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### 1. Introduction

Maize is one of the most commonly produced field crops. Its production area was 187.95 million hectares in 2018 and 1.06 billion tons was harvested worldwide (FAO, 2018). Twice the current grain harvest will be needed to meet the food needs of a population expected to grow to 9.6 billion by 2050 (Singh et al., 2016). The use of inorganic fertilizers is an important factor in successful maize production. Nitrogen is one of the most important nutrients that significantly affects plant height, leaf area, stem diameter, ear length and

thousand grain weight (Gruzka et al., 2016). While N-fertilization is essential to maintain and increase yields, it should be supplemented with alternative solutions to ensure environmentally friendly production (Aziz et al., 2019). Atmospheric nitrogen fixation by free-living and plant-associated bacteria contributes significantly to the environmentally friendly nitrogen supply to plants (Herridge et al., 2008). Photosynthesizing cyanobacteria were also used in rice cultivation due to their nitrogen fixation ability. However, this practice has ended in more developed countries as cyanobacteria are only able to sequester 20–30 kg/ha of nitrogen per year (Issa et al., 2014).

A promising avenue of research is the use of elicitor molecules that stimulate plant growth and help protect plants against biotic and abiotic stress (Singh, 2014). Biostimulants are organic substrates and microorganisms that when applied in small amounts to a plant, improve nutrient conversion, tolerance to abiotic stress and yield quality (du Jardin, 2015). As biostimulants increase yields without

Abbreviations: DW, dry weight; FW, fresh weight; GDD, growing days degree; GNC, grain nitrogen concentration; GY, grain yield; MACC, Mosonmagyaróvár Algae Culture Collection; RWC, relative water content; SW, saturation weight

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additional nutrient input, they play an important role in the development of environmentally sustainable technologies (Gortari et al., 2019). Biostimulants are grouped as humic substances, hormone-containing preparations and amino acid products. Extracts of marine macroalgae have been successfully used since the 1940s in agriculture to increase yields and improve the stress tolerance of crops (Craigie, 2011). The numerous positive physiological effects elicited with application of seaweed extracts is due to the presence of plant hormones (auxins, cytokinins, gibberellins and brassinosteroids) and other elicitor molecules such as betaines, polyamines and phlorotannins (reviewed in Stirk et al., 2020).

Similar to seaweeds, cyanobacteria and microalgae synthesize an array of endogenous hormones. For example, a number of endogenous cytokinins (including free bases, ribosides and monophosphates) and auxins (indole-3-acetic acid and amino acid conjugates) were identified in the cyanobacteria *Chroococcus minutus*, *Nostoc microscopium* and *Phormidium animale* (Žižková et al., 2017). Thus, there is potential to apply cyanobacteria to crops to improve their growth and yield. For example, when suspension cultures of *Nostoc entophyllum* were applied to winter rapeseed in a field trial, there was increased shoot growth (number of leaves and dry weight, longer shoot apices), increased root growth and increased chlorophyll content. The winter hardiness also improved in these more vigorous plants (Tóth et al., 2019). *Nostoc piscinale* applied to winter wheat in a three year field trial produced a stronger root system, elevated the relative water content (RWC) and chlorophyll content in the leaves and increased the proline content. This resulted in increased yield (ear number and length, grain number per ear and grain weight; Takács et al., 2019). When irrigation was stopped in a pot experiment, young sunflower plants treated with *Nostoc entophyllum* maintained their turgor one week longer than the control plants (Póthe et al., 2014).

Maize responds positively to foliar application of seaweed extracts (Jeannin et al., 1991; Basavaraja et al., 2018; Trivedi et al., 2018). Positive responses were also obtained with soil applications of cyanobacterial extracts (Grzesik and Romanovska-Duda, 2014; Prasanna et al., 2015; Dineshkumar et al., 2019) and with seed priming treatments (Sharma et al., 2020). However, most of these microalgae treated maize experiments were pot trials carried out under controlled conditions. The aim of the current experiment was to investigate the potential of foliar application of freeze-dried biomass of a cyanobacterium (*Nostoc piscinale*) resuspended in tap water to improve the growth, grain yield, yield components and stress tolerance of maize in a field trial in two regions in Hungary.

## 2. Material and methods

### 2.1. Experimental design

The experimental crop was the maize hybrid SY Zephir FA0390 (*Zea mays* L.). The field experiment was established in 2019 with identical treatments in two locations with different soil characteristics. Site 1 at the Széchenyi István University, Western Hungary (47° 52'N; 17°16'E) had alluvial soil. Site 2 at the University of Debrecen (47°33'N; 21°36'E) had calcareous chernozem soil formed on the Hajdúság loess-ridge. Soil characteristics of the two experiment sites are shown in Table 1. The experiment had a randomised block design with four replications (plots). Experimental plots were 100 m<sup>2</sup> in size that were part of a large maize field. Sowing was performed with 75 cm row spacing, 22 cm plant spacing and 6 cm sowing depth. A tefluthrin-containing soil disinfectant was applied at the time of sowing. Temperature and rainfall were monitored daily at each site.

The cyanobacterium MACC-612 *Nostoc piscinale* was obtained from the Mosonmagyaróvár Algae Culture Collection. To produce the biomass required for the experiments, the strain was grown in a previously described laboratory algae culture apparatus (Ördög, 1982) in Tamiya medium for 6 days, following the procedure described by

**Table 1**

Soil characteristics of the experimental field at the Mosonmagyaróvár Faculty Farm (Site 1) and at the Debrecen University Farm (Site 2) in the experimental year 2019.

Soil parameters	Site 1	Site 2
Soil type	Danubian alluvial soil	leached chernozem
Crop grown previously	winter barley	Maize
Humus content (m/m %)	3.20	3.70
Gold crown value	39	40
Liquid limit (K <sub>A</sub> )	52	46
pH KCl	7.2	7.5
Tilth (cm)	130	90
Salt (%)	0	0
CaCO <sub>3</sub> (m/m %)	21.4	11.4
Al-P <sub>2</sub> O <sub>5</sub> (mg/kg)	189	237
Al-K <sub>2</sub> O (mg/kg)	200	214
NO <sub>2</sub> -NO <sub>3</sub> -N (mg/kg)	12.8	24.3

Takács et al. (2019). The resulting biomass was centrifuged at 2150 g for 15 min at room temperature and then freeze-dried. The biomass was stored at -18 °C until required.

The freeze-dried biomass was suspended in tap water (1 g/L DW) before treatment and sonicated for 3 min (VirTis, VirSonic 600 Ultrasonic Cell Disruptor). The 6-7-leaf maize plants (V6-V7 phenological stage) were sprayed with 400 L/ha cyanobacterial suspension. This concentration was selected based on results from field trials with winter wheat (Takács et al., 2019) and rapeseed (Tóth et al., 2019). For better adhesion to the leaf, Trend 90 was used as a wetting agent on Site 1 and ethoxylated isodecyl alcohol (0.1%) on Site 2. Control plots at both field sites were treated with tap water containing only the wetting agent. The application was performed with a manual sprayer at a temperature below 25 °C. The Darby-Lauer (2004) scale was used to determine maize phenological phases. On-site measurements and sampling for laboratory analyses were performed on plants in the middle rows of the plots. The dates of sowing, treatment and harvesting are shown in Table 2. The plots of the experimental plots were not irrigated and fertilized during the vegetation period.

The daily temperature readings were used to calculate heat units. The heat sums of the different phenological stages of maize were calculated from temperatures above 10 °C and below 30 °C.

### 2.2. Plant growth parameters

New leaves appearing on the control and *N. piscinale* treated plants, as well as changes in leaf number were recorded weekly during the growing season. Prior to harvest, 40 whole ear samples were collected per plot. Cob length and diameter as well as the number of grain rows were counted. The grains were removed from the cob and dried for 72 h at 60 °C to a constant weight and then weighed. The grain yield was calculated from the yield per pot and reported at 14% moisture content.

### 2.3. Chlorophyll content

The chlorophyll concentration of the second and third youngest leaves were determined with the SPAD 502 Plus Chlorophyll Meter (MINOLTA, Japan) portable device which is widely used to estimate foliar chlorophyll content in a non-destructive way (Kalaji et al., 2017; Vesali et al., 2017). These measurements commenced one week after the *N. piscinale* treatment and were carried out weekly during the vegetation period, always around 4 pm. Measurements were taken from 5 plants/plot. The chlorophyll content was expressed in SPAD units.

### 2.4. Relative water content of leaves

The relative water content (RWC %) of the second and third youngest leaves were measured weekly (3 plants/plot) using the method

**Table 2**

Sowing, treatment and harvest dates of maize grown at the Mosonmagyaróvár Faculty Farm (Site 1) and at the Debrecen University Farm (Site 2). Treatment is the date SY Zephir maize hybrid was treated with *N. piscinale* suspension (1 g/L) applied as a foliar spray (400 L/ha) at the phenological stage V6-V7.

	Site 1	Site 2
Sowing time	30.04.2019	03.05.2019
Treatment	10.06.2019	07.06.2019
Harvest time	14.10.2019	10.09.2019

of Cabrera-Bosquet et al. (2009), starting one day before the *N. piscinale* treatment. The weight of the freshly cut leaves (fresh weight, FW) was measured, immersed in water for 24 h (saturated weight, SW) and then oven dried (60 °C) (dry weight, DW) for 24 h. The relative water content was calculated using the following formula:  $RWC\% = (FW - DW) / (SW - DW) \times 100$ .

### 2.5. Proline content

The proline content of the second and third youngest leaves (0.3 g FW) was quantified using the method of Bates et al. (1973). Measurements started one day before the *N. piscinale* treatment and were carried out once a week for the duration of the field trials using 4 plants/plot. The proline concentration ( $\mu\text{g/mL}$ ) was measured from a standard curve and the proline content of the biomass calculated on a fresh weight basis as follows:  $[(\mu\text{g proline/mL} \times 3 \text{ mL toluene}) / 115.5 \mu\text{g}/\mu\text{mol}] / [0.3 \text{ g sample}/5] = \mu\text{mol proline/g FW weight}$ .

### 2.6. Raw protein content

The protein content of the grain yield was determined using the Dumas combustion method (Ebeling, 1968). Samples were subjected to oxidative digestion at a high temperature (approximately 900 °C) with a controlled oxygen supply. The resulting flue gases passed through a copper oxide-platinum catalyst using a CO<sub>2</sub> carrier gas, thus ensuring complete oxidation. After the subsequent reduction processes and the purification of the carrier gas, the nitrogen content remaining in the CO<sub>2</sub> carrier gas was detected in a thermal conductivity detector (VELP NDA 702, Velp Scientific, Italy). The N<sub>2</sub> volume provided an electrical measurement signal from which the nitrogen content of the various burned samples and the protein content derived from them was measured and calculated automatically on the basis of the pre-prepared calibration curve. The analysis was carried out in 4 replicates.

### 2.7. Statistical analysis

The obtained results were analysed using Dell Statistica 13.2 and Microsoft Excel<sup>R</sup> 2010. The standard deviations (Student test) of the 4 replicates (plots) within each treatment and the significant difference between the control and each treatment was calculated using Duncan's test. Bold numbers in the tables indicate that the values are statistically different from the control.

## 3. Results

### 3.1. Precipitation and temperature

The meteorological data of the two field sites are shown in Fig. 1. Precipitation during the field trial was higher on Site 1 (347 mm) than on Site 2 (279.2 mm). Higher precipitation fell during the vegetative growth phase at Site 1 compared to Site 2. During the reproductive stage, the amount of precipitation was similar at both sites.

The average temperature was similar at both sites (18.1 °C and 17.6 °C). In July–August, the amount of precipitation required for the ideal development and yield formation of maize on Site 1 was low (68 mm) and the average monthly air temperature was high (22.2 °C in July and 22.6 °C in August). During the reproductive stage of maize, the number of days with unfavourable temperatures above 30 °C was 27 days at Site 1. Sufficient (114 mm) precipitation fell at Site 2 in July–August with the average temperature remaining favourably low in July (20.5 °C) but high in August (22.2 °C). During the reproductive stage of maize, the number of days with temperatures above 30 °C was 20 at Site 2.

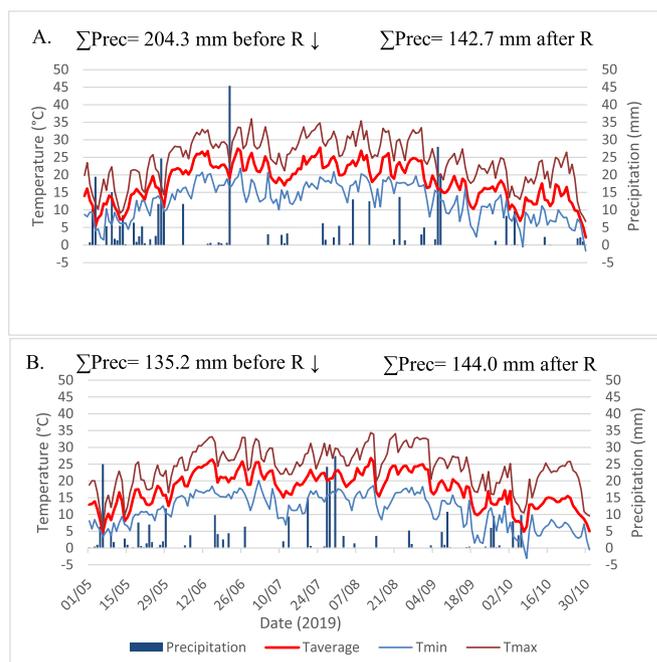
### 3.2. Heat sum and number of leaves

Based on heat sums, the initial development of maize up to the 6–7 leaf stage (V6–V7) was similar at both field sites (Table 3). Following the *N. piscinale* treatment, the different phenological stages characterized by leaf number in the vegetative stages differed over time in the control and *N. piscinale* treated plants. At both production sites, at the heat sum value of 300–309, the number of leaves of the *N. piscinale* treated plants was 10 compared to the 9 leaves of the control plants and by V11 (Site 1) and V12 (Site 2), the difference in leaf number was significant. This difference persisted until silking (R1; Table 3). Thus the development of the *N. piscinale* treated maize plants was faster in the vegetative stage than that of the control plants at both production sites.

Development was similar after silking (R1–R6), but 211 °C more heat was required after silking (R1) to reach physiological maturity (R6) on Site 1 compared to Site 2 (Table 3).

### 3.3. Chlorophyll content of leaves

At Site 1, the trend of SPAD values of the second and third youngest leaves was similar during the vegetative period. Values ranged from 45 to 50 until the V12–V13 phenological stage, increased rapidly to 60–65 until female flowering (R1) and then continuously



**Fig. 1.** Average (Taverage), maximum (Tmax) and minimum (Tmin) air temperature and precipitation at Site 1 Széchenyi István University (A) and Site 2 University of Debrecen (B) during the field trial in 2019. Blue columns show the daily precipitation (mm).  $\Sigma\text{Prec}$  is the sum of precipitation before and after the start of the reproductive growth phase (R) of the maize hybrid.

**Table 3**

Leaf number of control and *N. piscinale* treated maize hybrid plants at different phenological phases and the sum of daily heat units during the field trial in 2019 at the Mosonmagyaróvár Faculty Farm (Site 1) and the Debrecen University Farm (Site 2). Plants were treated with the cyanobacterium suspension (1 g/L) applied at 400 L/ha at the phenological stages V6-V7. ns: no significant difference; p\*: significant difference ( $p \leq 0.05$ ) between the control and treated plants at each site and phenological stage ( $n = 10$ ).

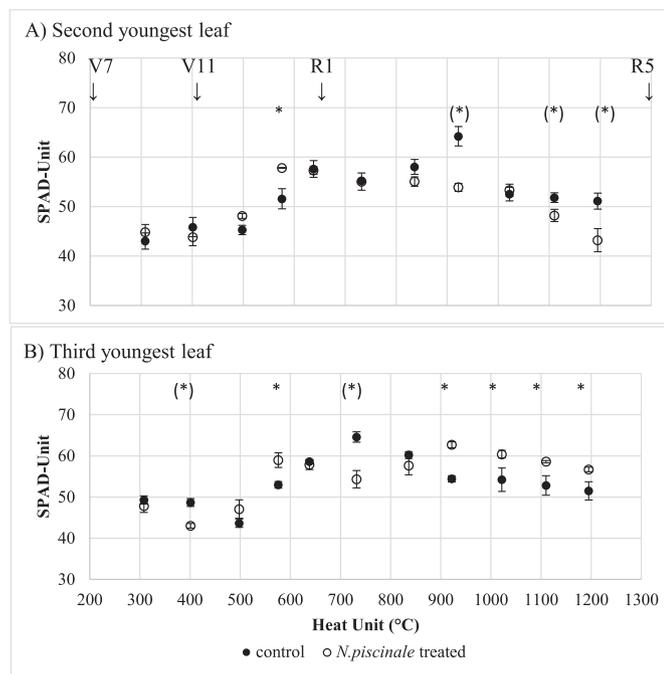
Sampling Date (2019)	Leaf number							
	Site 1				Site 2			
	Heat unit (°C)	Phenological stage	Control	<i>N. piscinale</i> treated	Heat unit (°C)	Phenological stage	Control	<i>N. piscinale</i> treated
21.05	21	V3	3	3 <sub>ns</sub>	31	V2	2	2 <sub>ns</sub>
28.05	65	V4	4	4 <sub>ns</sub>	70	V3	3	3 <sub>ns</sub>
04.06	121	V6	6	6 <sub>ns</sub>	126	V4	4	4 <sub>ns</sub>
11.06	208	V7	7	7 <sub>ns</sub>	206	V7	7	7 <sub>ns</sub>
18.06	309	V9	9	10 <sub>ns</sub>	300	V9	9	10 <sub>ns</sub>
25.06	401	V11	11	12 <sub>p*</sub>	383	V11	11	11 <sub>ns</sub>
02.07	499	V13	12	14 <sub>p*</sub>	471	V12	12	14 <sub>p*</sub>
09.07	577	V14	13	15 <sub>p*</sub>	545	V14	14	16 <sub>p*</sub>
16.07	639	VT	14	16 <sub>p*</sub>	591	VT	15	17 <sub>p*</sub>
23.07	733	R1	15	17 <sub>p*</sub>	665	R1	16	17 <sub>ns</sub>
30.07	836	R2	17	17 <sub>ns</sub>	762	R2	17	17 <sub>ns</sub>
13.08	1023	R3	18	18 <sub>ns</sub>	923	R4	18	18 <sub>ns</sub>
27.08	1195	R4	18	18 <sub>ns</sub>	1085	R5	18	18 <sub>ns</sub>
10.09	1337	R5	18	18 <sub>ns</sub>	1250	R6	18	18 <sub>ns</sub>
14.10	1461	R6	18	18 <sub>ns</sub>	-	-	-	-

decreased until the end of the reproductive stage (Fig. 2). *N. piscinale* treated plants had significantly different SPAD values, especially during reproductive stage. SPAD values were lower in the second youngest leaf of the *N. piscinale* treated plants (Fig. 2A) but higher in the third leaf compared to the control plants (Fig. 2B).

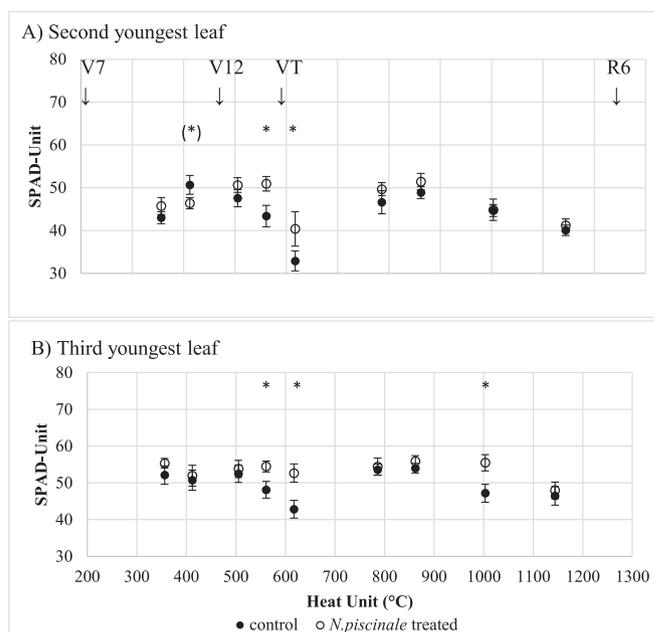
At Site 2, SPAD values mostly varied between 45 and 50 for the second youngest leaf and 50–55 for the third leaf. SPAD values decreased at the end of the vegetative and reproductive stages to 40–45 for both leaves (Fig. 3). SPAD values were higher in the *N. piscinale* treated plants compared to the control plants with significant differences during the vegetative stages (Fig. 3).

3.4. Relative water content of leaves

The RWC of maize grown on Site 1 ranged from 54% to 71% and on Site 2 from 73% to 90%. On Site 1, the RWC in the second and third youngest leaves of maize was consistently higher in *N. piscinale* treated plants compared to the control plants. The RWC was between 5.7 and 10.4% higher for the second youngest leaf and between 2.5 and 15.4% for the third leaf. However, this was only statistically significant in the second leaf at the phenological stage V9-V10 and in the third leaf at V14 and R2-R3 stages (Table 4). At Site 2, the RWC of the leaves of the *N. piscinale* treated plants were lower than or similar to the RWC of the control plants, with one exception (second leaf at V9-V10 significantly different). The differences were only significant



**Fig. 2.** Relative chlorophyll content (SPAD-unit) of the A) second and B) third youngest leaves of the SY Zephir maize hybrid at Site 1 (Széchenyi István University). Measurements were taken weekly at 4 pm and started one day before the *N. piscinale* treatment (1 g/L) applied at 400 L/ha. Averages of 24 SPAD values (6 plants per 4 replicates) are plotted against the heat sums. Significant differences ( $p \leq 0.05$ ) are shown as \* for values higher than the control and (\*) for values lower than the control.



**Fig. 3.** Relative chlorophyll content (SPAD-unit) of the A) second and B) third youngest leaves of the SY Zephir maize hybrid at Site 2 (University of Debrecen). Measurements were taken weekly at 4 pm and started one day before the *N. piscinale* treatment (1 g/L) applied at 400 L/ha. Averages of 24 SPAD values (6 plants per 4 replicates) are plotted against the heat sums. Significant differences ( $p \leq 0.05$ ) are shown as \* for values higher than the control and (\*) for values lower than the control.

**Table 4**

RWC of the second and third youngest maize leaf in control and *N. piscinale* treated maize plants at the two field sites. Different letters in each column indicate significant differences. \* ( $p \leq 0.05$ ) and \*\* ( $p \leq 0.01$ ) indicates significant difference between the control and treated plants at each site and phenological stage ( $n = 24$ ).

Phenological stage	RWC (%)			
	Second youngest leaf		Third youngest leaf	
	Control	<i>N. piscinale</i> treated	Control	<i>N. piscinale</i> treated
Site 1				
V9-10	54.8 ± 1.5 <sup>c</sup>	<b>60.5 ± 2.5<sup>a</sup></b>	56.6 ± 0.9 <sup>a</sup>	58.5 ± 0.8 <sup>b</sup>
V11-12	54.2 ± 1.1 <sup>c</sup>	62.4 ± 3.9 <sup>a</sup>	59.7 ± 0.5 <sup>a</sup>	61.2 ± 2.5 <sup>b</sup>
V14	60.0 ± 2.1 <sup>b</sup>	65.3 ± 0.5 <sup>a</sup>	60.8 ± 0.5 <sup>a</sup>	<b>70.2 ± 3.1<sup>a</sup></b>
R1	61.0 ± 1.1 <sup>b</sup>	65.0 ± 0.4 <sup>a</sup>	63.4 ± 1.3 <sup>a</sup>	68.6 ± 0.7 <sup>a</sup>
R2-3	61.1 ± 2.1 <sup>b</sup>	64.5 ± 1.0 <sup>a</sup>	65.0 ± 1.0 <sup>a</sup>	<b>68.5 ± 0.5<sup>a</sup></b>
R3-4	69.0 ± 1.6 <sup>a</sup>	69.5 ± 0.6 <sup>a</sup>	67.6 ± 2.2 <sup>a</sup>	71.2 ± 1.1 <sup>a</sup>
Site 2				
V9-10	80.9 ± 2.5 <sup>b</sup>	<b>83.2 ± 1.6<sup>**a,b</sup></b>	82.2 ± 4.17 <sup>a,b</sup>	80.8 ± 4.9 <sup>b</sup>
V12	89.5 ± 3.0 <sup>a</sup>	86.7 ± 1.8 <sup>a</sup>	87.9 ± 3.7 <sup>a</sup>	86.0 ± 2.1 <sup>a</sup>
V13	83.1 ± 3.1 <sup>a,b</sup>	82.6 ± 2.2 <sup>a,b</sup>	80.3 ± 1.8 <sup>b</sup>	80.4 ± 3.9 <sup>b</sup>
VT	88.4 ± 5.0 <sup>a</sup>	88.4 ± 1.1 <sup>a</sup>	90.0 ± 1.6 <sup>a</sup>	88.9 ± 1.6 <sup>a</sup>
R2	78.2 ± 3.5 <sup>b</sup>	75.5 ± 0.7 <sup>c</sup>	76.3 ± 3.0 <sup>b</sup>	76.1 ± 1.6 <sup>b,c</sup>
R5	80.1 ± 1.3 <sup>b</sup>	<b>75.1 ± 2.9<sup>***c</sup></b>	80.6 ± 2.1 <sup>b</sup>	<b>73.4 ± 1.7<sup>***c</sup></b>

in the V9-V10 of the second leaf and the R5 phenological stage of the second and third leaves (Table 4).

### 3.5. Free proline content

The trend in all the maize plants was for the proline content in the second and third youngest leaves to gradually decreased over the course of the field trial with a sharp decline after the R1 and/or VT phenological stage (Table 5). At both field sites, the proline content of the second and third youngest leaves of the *N. piscinale* treated plants was always higher than in the control plants. However, this difference was only significant at the R1 phenological stage of the third leaf on Site 1 and at the R5 stage of the third leaf on Site 2 (Table 5).

### 3.6. Grain yield, yield components and protein content

Grain yield increased significantly by 6.5% on Site 1 and by 11.5% on Site 2 as a result of the *N. piscinale* treatment. Overall, grain yield

was higher at Site 2 compared to Site 1. The protein content of the grain also increased due to the *N. piscinale* treatment on both production sites but was only significant at Site 1 (Table 6). Thousand grain weight also increased significantly with *N. piscinale* treatment at both production sites along with an increase in cob length (Site 1) and cob diameter (Site 2; Table 6).

## 4. Discussion

The effectiveness of biostimulant treatments varies with plant species and variety and the method and time of application. A single foliar application of *N. piscinale* suspension had a beneficial effect on maize growth at both production sites in the present study. The first visible sign of the effect of the cyanobacterial treatment was an increase in leaf number where there were 1–2 extra leaves on the treated plants compared to the control plants between phenological phases V9 and R1 (Table 3). More leaves are associated with a larger leaf area, increased light absorption and higher photosynthetic

**Table 5**

Free proline content of the second and third youngest maize leaf in the control and *N. piscinale* treated plants grown at the two field sites. Different letters in each column indicate significant differences. \* ( $p \leq 0.05$ ) and \*\* ( $p \leq 0.01$ ) indicates significant difference between the control and treated plants at each site and phenological stage ( $n = 4$ ).

Phenological stage	Proline content ( $\mu\text{mol/g FW}$ )			
	Second youngest leaf		Third youngest leaf	
	Control	<i>N. piscinale</i> treated	Control	<i>N. piscinale</i> treated
Site 1				
V9-10	12.96 ± 1.66 <sup>a</sup>	13.93 ± 0.56 <sup>a</sup>	12.84 ± 2.26 <sup>a</sup>	14.18 ± 0.75 <sup>a</sup>
V11-12	7.56 ± 0.56 <sup>b</sup>	8.27 ± 0.61 <sup>b</sup>	7.42 ± 0.61 <sup>b</sup>	8.47 ± 1.60 <sup>b</sup>
V14	6.12 ± 2.07 <sup>c</sup>	7.28 ± 1.93 <sup>b</sup>	6.80 ± 2.54 <sup>b</sup>	8.22 ± 0.46 <sup>b</sup>
R1	4.73 ± 0.75 <sup>d</sup>	5.28 ± 0.87 <sup>c</sup>	4.18 ± 0.65 <sup>c</sup>	<b>6.61 ± 0.86<sup>**c</sup></b>
R2-3	0.96 ± 0.36 <sup>f</sup>	1.34 ± 0.32 <sup>d,e</sup>	1.09 ± 0.24 <sup>d</sup>	1.28 ± 0.47 <sup>d</sup>
R3-4	1.15 ± 0.22 <sup>e</sup>	1.41 ± 0.46 <sup>e</sup>	1.11 ± 0.45 <sup>d</sup>	1.44 ± 0.38 <sup>d</sup>
R4	0.72 ± 0.11 <sup>f</sup>	0.84 ± 0.08 <sup>e</sup>	0.88 ± 0.09 <sup>d</sup>	0.82 ± 0.10 <sup>d</sup>
Site 2				
V9-10	12.10 ± 2.02 <sup>a</sup>	12.71 ± 2.04 <sup>a</sup>	14.57 ± 2.28 <sup>a</sup>	15.97 ± 2.34 <sup>a</sup>
V12	10.40 ± 1.55 <sup>a,b</sup>	10.63 ± 1.99 <sup>a,b</sup>	9.31 ± 1.66 <sup>b</sup>	9.99 ± 1.11 <sup>b</sup>
V13	7.23 ± 1.66 <sup>c</sup>	7.58 ± 1.54 <sup>c</sup>	7.23 ± 1.60 <sup>c</sup>	8.22 ± 2.09 <sup>b,c</sup>
VT	5.57 ± 1.51 <sup>d</sup>	6.20 ± 1.54 <sup>c,d</sup>	5.91 ± 1.90 <sup>c,d</sup>	6.53 ± 1.70 <sup>c</sup>
R2	2.27 ± 0.43 <sup>e</sup>	3.32 ± 0.84 <sup>e</sup>	3.12 ± 0.62 <sup>d,e</sup>	3.82 ± 0.99 <sup>d</sup>
R3	1.12 ± 0.32 <sup>f</sup>	1.82 ± 0.57 <sup>f</sup>	1.93 ± 0.59 <sup>e</sup>	2.02 ± 0.61 <sup>d,e</sup>
R5	1.02 ± 0.22 <sup>f</sup>	1.36 ± 0.37 <sup>f</sup>	0.91 ± 0.38 <sup>f</sup>	<b>1.13 ± 0.29<sup>**e</sup></b>

**Table 6**

The effect of the *N. piscinale* treatment on grain yield, yield components and the yield protein content of maize hybrid SY Zephir FAO390 from both field sites. Different letters in each row indicate significant differences between treatments and site where \*\* $p \leq 0.01$  and \*\*\* $p \leq 0.001$  ( $n = 5$  at Site 1,  $n = 4$  at Site 2).

	Site 1		Site 2	
	Control	<i>N. piscinale</i> treated	Control	<i>N. piscinale</i> treated
Grain yield (kg/ha)	8140 ± 22 <sup>a</sup>	<b>8675 ± 24<sup>c***</sup></b>	10742 ± 37 <sup>b</sup>	<b>11978 ± 41<sup>d**</sup></b>
Grain protein content (%)	7.8 ± 0.2 <sup>a</sup>	<b>9.3 ± 0.3<sup>c***</sup></b>	8.7 ± 1.8 <sup>b</sup>	9.4 ± 1.2 <sup>c</sup>
Grain-cob ratio	14.28 ± 2.00	16.48 ± 0.81	16.7 ± 4.03	16.5 ± 3.63
Thousand grain weight (g)	190.08 ± 0.1 <sup>b</sup>	203.60 ± 0.2 <sup>***</sup>	236.13 ± 0.6 <sup>b</sup>	260.33 ± 0.7 <sup>***</sup>
Cob length (cm)	22.0 ± 0.4 <sup>b</sup>	23.6 ± 0.4 <sup>a**</sup>	20.26 ± 0.5 <sup>a</sup>	19.66 ± 0.3 <sup>b</sup>
Cob diameter (cm)	6.5 ± 0.2 <sup>a</sup>	6.2 ± 0.2 <sup>b</sup>	4.7 ± 0.1 <sup>b</sup>	4.9 ± 0.1 <sup>a</sup>
Number of grain rows	17.3 ± 0.5 <sup>a</sup>	16.8 ± 0.5 <sup>b</sup>	17.0 ± 1.2 <sup>b</sup>	17.5 ± 1.9 <sup>a</sup>

organic matter production which would be beneficial to the crop. Similarly, in a pot trial with maize, *Chlorella vulgaris* and *Spirulina platensis* (applied at 3 g/kg soil) increased the leaf number from 6 leaves in control plants to 8 leaves in the treated plants twenty days after sowing (Dineshkumar et al., 2019). However, larger leaf area leads to greater transpiration, more rapid water loss and leaf roll in the event of water deficit stress. This can be fatal for photosynthesis and nitrogen uptake (Wang et al., 2020). In the present study, no leaf roll was observed at either production site.

The economics of agricultural production is significantly affected by temperature and precipitation. Maize hybrids respond strongly to temperature changes and are less dependent on photoperiod (Moeletsi, 2017). For maize, temperatures above 10 °C and below 30 °C are favourable although the optimum temperature varies depending on the phenological stage. Maize is particularly sensitive to temperatures above 30 °C during silking and grain filling with high temperatures reducing pollen count and viability as well as grain weight (Butler and Huybers, 2015; Deng et al., 2019). In the present experiment, Site 1 had a maximum daily temperature (Tmax) above 30 °C for 27 days and the rainfall was only 68 mm after silking, which adversely affected the yield production (Table 6). In contrast, on Site 2, Tmax was above 30 °C for only 20 days and precipitation was sufficient (114 mm; Fig. 1). Higher precipitation during this vegetative period on Site 2 was reflected in higher RWC values while lower precipitation on Site 1 was reflected in lower RW values (Table 4).

Plants need a certain amount of heat accumulated during the growing season in order to grow and produce yield. While growth is influenced by the combined effects of several environmental factors, the development of the plant is directly affected by temperature changes. Growth of maize is halted below 10 °C but can develop from one phenological stage to the next if it receives its heat demand in the previous stage (Moeletsi, 2017). Crop growers most often use the GDD (Growing Days Degree) index to calculate the heat sum (Grigorieva, 2020). Due to the better water holding capacity and higher soil-N content on Site 2 (Table 1) and the more favourable rainfall distribution, the plants developed faster and required less heat sum (211 °C) for physiological maturation than on Site 1 (Table 3).

The chlorophyll content of leaves, which can be characterized by SPAD values, is an easily measurable and useful stress indicator (Arif et al., 2018). There is a strong correlation between the total nitrogen and chlorophyll content of leaves and it therefore reflects the nitrogen status of the leaf (Hirel et al., 2005). Nitrogen is a component of chlorophyll and photosynthetic enzymes and is necessary for their synthesis. It thus directly and indirectly affects plant photosynthesis and as a result, plant yield (Wang et al., 2020). Nitrogen accumulated in the grain originates from N uptake after silking and from the mobilization of nitrogen accumulated in the vegetative organs during the preceding period (Hirel et al., 2007). A high proportion (50–70%) of the leaf nitrogen content is mobilized (Chen et al., 2014, 2015a, 2016), mostly originating from photosynthetic enzymes and less from chlorophyll and nitrogen in the thylakoids. This results in a

decrease in photosynthesis in the later stages of grain filling (Mu et al., 2018). The decrease in leaf nitrogen content begins about 15 days after silking (Ning et al., 2017). This N mobilization to the grains was reflected in the decreasing SPAD values in the reproductive stage of maize development in the current study (Figs. 2 and 3).

The water supply of the plant affects the chlorophyll content of the leaf with concentrations increasing during rainy periods and decreasing during dry periods (Khayatnezhad and Gholamin, 2012; Shafiq et al., 2019). In the present study, there were fluctuations in the chlorophyll content of the leaves throughout the vegetative stage on both production sites with higher SPAD values for the third leaf compared to the second leaf (Figs 2 and 3). The cyanobacterial treatment affected the chlorophyll content during the reproductive stages at Site 1 but had less effect at Site 2. SPAD values were significantly higher in the third leaves in *N. piscinale* treated plants at Site 1, allowing for greater photosynthetic light absorption over a longer period of time. This indicated that a single cyanobacterial foliar treatment improved the plants tolerance to the less favourable environmental conditions (higher temperatures and lower precipitation) occurring at Site 1.

The water content of the leaf affects many plant physiological processes. Water shortage significantly reduces plant weight and affects crop yield (Arndt et al., 2015). The RWC of leaves is an important indicator of the water status of plants, reflecting the rate of leaf water uptake and transpiration (Lugojan and Ciulca, 2011). In the present study, the cyanobacterium treatment did not significantly affect the RWC of the hybrid maize leaves at either production site, except for a few measurement dates. Higher RWC values (73–90%) were measured in plants grown at Site 2 than Site 1 (54–71%) (Table 4) and this contributed to the higher yields achieved at Site 2 (Table 6).

Accumulation of proline occurs under abiotic and biotic stress as proline has a protective role against stress (Szabados and Savouré, 2009). The free proline content is suitable parameter for evaluating drought stress tolerance in plants. Under dry conditions, a higher proline content is associated with greater drought stress tolerance and relatively high yields in maize (Shafiq et al., 2019). The relatively stable RWC during the vegetation period and the lack of leaf roll suggest that no significant water shortage stress developed at either production site in the present study and hence there was no increase in proline concentrations. With the exception of two measurements, the proline content was not significantly affected by the *N. piscinale* treatment (Table 5). In contrast, *N. piscinale* applied during tillering increased the proline content in wheat leaves 2-3-fold compared to control plants (Takács et al., 2019).

The proline content of the leaves decreased steadily throughout the vegetative and reproductive stages in the hybrid maize with a sharp drop at the start of the reproductive stage at both experimental sites (Table 5). Proline, in addition to its role in cellular protein synthesis and response to environmental stress effects, acts as a signalling molecule throughout the reproductive stage of maize from flowering to grain filling (Mattioli et al., 2009; Szabados and Savouré,

2009; Wang et al., 2020). Proline also provides energy to maintain the energy-intensive metabolism of plant reproduction. Oxidation of a proline molecule provides the energy equivalent to 30 ATPs (Atkinson, 1977). There is upregulation of genes involved in the breaking down of proline in flowers and seeds in order to provide sufficient energy throughout the reproductive stages (Atkinson, 1977). Similarly, there was an initial high proline content and a decrease in its concentration during the vegetation period in wheat (Takács et al., 2019).

Important parameters in maize production is grain yield (GY) and the grain nitrogen concentration (GNC). Nitrogen and genotype are the two main factors affecting GY and GNC in maize (Chen et al., 2015a,b). Due to the more favourable soil and environmental conditions at Site 2, the yield of the control plots increased by 32% compared to Site 1 in the present experiment. A single foliar application of *N. piscinale* suspension significantly increased the grain yield of the SY Zephir maize hybrid at both production sites (6.5% at Site 1 and 11.5% at Site 2) (Table 6). Grain yield was significantly influenced by cob size and thousand grain weight (Table 6). The beneficial effects on the cyanobacterium treatment on the grain harvest can be attributed to the biostimulatory effect on vegetative growth (leaf number) and an increased chlorophyll content during the reproductive stages, allowing for greater photosynthetic light absorption over a longer period of time. A similar increase in yield was obtained when a seaweed based biostimulant was applied to maize at various phenological stages. The highest yield increases were obtained with applications at V5, V15 and V5 + V15 (13.7%, 11.1% and 32% yield increase, respectively; Trivedi et al., 2017).

In conclusion, the results of the field trials conducted in two different regions in Hungary proved that the use of cyanobacterium-based biostimulants can contribute to crop production and yield increase in a sustainable and environmentally friendly manner. In the present study, a single foliar application of a *N. piscinale* suspension significantly increased growth and grain yield in maize grown under different production conditions.

## Declaration of Competing Interest

None.

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## References

Arif, T.U., Siddiqui, N., Sohag, A.A.M., Sakil, A., Rahman, M., Polash, M.A.S., Mostofa, M.G., Tran, L.S.P., 2018. Salicylic acid-mediated enhancement of photosynthesis attributes and antioxidant capacity contributes to yield improvement of maize plants under salt stress. *J. Plant Growth Regul.* 37, 1318–1330.

Arndt, S.K., Irawana, A., Sanders, G.J., 2015. Apoplastic water fraction and rehydration techniques introduce significant errors in measurements of relative water content and osmotic potential in plant leaves. *Physiol. Plant.* 155, 355–368.

Atkinson, D.E., 1977. *Cellular Energy Metabolism and its Regulation*. Academic Press, New York.

Aziz, M.Z., Naveed, M., Abbas, T., Siddique, S., Yaseen, M., 2019. Alternative fertilizers and sustainable agriculture. In: Farooq, M., Pisante, M. (Eds.), *Innovations in Sustainable Agriculture*. Springer, Cham, pp. 213–245.

Basavaraja, P.K., Yogendra, N.D., Zodape, S.T., Prakash, R., Ghosh, A., 2018. Effects of seaweed sap as foliar spray on growth and yield of hybrid maize. *J. Plant Nutr.* 41, 1851–1861.

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water stress studies. *Plant Soil* 39, 205–207.

Butler, E.E., Huybers, P., 2015. Variations in the sensitivity of US maize yield to extreme temperatures by region and growth phase. *Environ. Res. Lett.* 10, 1–8.

Cabrera-Bosquet, L., Molero, G., Nogues, S., Araus, J.L., 2009. Water and nitrogen conditions affect the relationships of  $\Delta^{13}\text{C}$  and  $\Delta^{18}\text{O}$  to gas exchange and growth in durum wheat. *J. Exp. Bot.* 60, 1633–1644.

Chen, Y., Xiao, C., Chen, X., Zhang, Q.L., Chen, F., Yuan, L., Mi, G., 2014. Characterization of the plant traits contributed to high grain yield and high grain nitrogen concentration in maize. *Field Crops Res.* 159, 1–9.

Chen, K., Kumudini, S.V., Tollenaar, M., Vyn, T.J., 2015a. Plant biomass and nitrogen partitioning changes between silking and maturity in newer versus older maize hybrids. *Field Crops Res.* 183, 315–328.

Chen, Y., Xiao, C., Wu, D., Xia, T., Chen, Q., Chen, F., Yuan, L., Mi, G., 2015b. Effects of nitrogen application rate on grain yield and grain nitrogen concentration in two maize hybrids with contrasting nitrogen remobilization efficiency. *Eur. J. Agron.* 62, 79–89.

Chen, Q., Mu, X., Chen, F., Yuan, L., Mi, G., 2016. Dynamic change of mineral nutrient content in different plant organs during the grain filling stage in maize grown under contrasting nitrogen supply. *Eur. J. Agron.* 80, 137–153.

Craigie, J.S., 2011. Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.* 23, 371–393.

Darby, H., Lauer, J., 2004. Plant physiology—critical stages in the life of a corn plant. *Field Corn* 17–24 (online) <http://www.mn.nrcs.usda.gov/technical/ecs/pest/planningaids> (accessed January 5, 2007).

Deng, X., Huang, Y., Sun, W., Yu, L., Hu, X., Wang, S., 2019. Different time windows provide divergent estimates of climate variability and change impacts on maize yield in Northeast China. *Sustainability* 11, 1–17.

Dineshkumar, R., Subramanian, J., Gopalsamy, J., Jayasingam, P., Arumugam, A., Kannadasan, S., Sampathkumar, P., 2019. The impact of using microalgae as biofertilizer in maize (*Zea mays* L.). *Waste Biomass Valorization* 10, 1101–1110.

du Jardin, P., 2015. Plant biostimulants: definition, concept, main categories and regulation. *Sci. Hortic.* 196, 3–14.

Ebeling, M.E., 1968. The Dumas method for nitrogen in feeds. *J. Assoc. Off. Anal. Chem.* 51, 766–770.

FAO, 2018. *Food and Agricultural Organization, FAO Yearbook Production. Food and Agricultural Organization of the United Nations*, 54, p. 115. Rome.

Gortari, F., Nowosad, M.I.P., Laczkeski, M.E., Onetto, A., Cortese, I.J., Castrillo, M.L., Bich, G.A., Alvarenga, A.E., Lopez, A.C., Villalba, L., Zapata, P.D., Rocha, P., Niella, F., 2019. Biofertilizers and biocontrollers as an alternative to the use of chemical fertilizers and fungicides in the propagation of Yerba Mate by mini-cuttings. *Revista Árvores* 43, 412–430.

Grigorieva, E., 2020. Evaluating the sensitivity of growing degree days as an agro-climatic indicator of the climate change impact: a Case Study of the Russian Far East. *Atmosphere* 11, 1–22.

Gruzka, M., Ohse, S., Pereira, A.B., Dias, C.T.D.S., 2016. Corn yield as a function of amounts of nitrogen applied in bands. *Afr. J. Agric. Res.* 11, 1805–1814.

Grzesik, M., Romanowska-Duda, Z., 2014. Improvements in germination, growth, and metabolic activity of corn seedlings by grain conditioning and root application with cyanobacteria and microalgae. *Pol. J. Environ. Stud.* 23, 1147–1153.

Herridge, D.F., Peoples, M.B., Boddey, R.M., 2008. Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil* 311, 1–18.

Hirel, B., Andrieu, B., Valadier, M.H., Renard, S., Quillere, I., Chelle, M., Pommel, B., Fournier, C., Drouet, J.L., 2005. Physiology of maize II: Identification of physiological markers representative of the nitrogen status of maize (*Zea mays*) leaves during grain filling. *Physiol. Plant.* 124, 178–188.

Hirel, B., Le Gouis, J., Ney, B., Gallais, A., 2007. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J. Exp. Bot.* 58, 2369–2387.

Issa, A.A., Abd-Alla, M.H., Ohyama, T., 2014. Nitrogen fixing cyanobacteria: future prospect. In: Ohyama, T. (Ed.), *Advances in Biology and Ecology of Nitrogen Fixation*. InTechOpen 2, Rijeka.

Jeannin, I., Lescure, J.C., Morot-Gaudry, J.F., 1991. The effects of aqueous seaweed spray on the growth of maize. *Bot. Mar.* 34, 469–473.

Kalaji, H.M., Dabrowski, P., Cetner, M.D., Samborska, I.A., Lukasik, I., Brestic, M., Zivcak, M., Tomasz, H., Mojski, J., Kociel, H., Panchal, B.M., 2017. A comparison between different chlorophyll content meters under nutrient deficiency conditions. *J. Plant Nutr.* 40, 1024–1034.

Khayatnezhad, M., Gholamin, R., 2012. The effect of drought stress on leaf chlorophyll content and stress resistance in maize cultivars (*Zea mays*). *Afr. J. Microbiol. Res.* 6, 2844–2848.

Lugojan, C., Ciulca, S., 2011. Evaluation of relative water content in winter wheat. *J. Hortic. Forest. Biotechnol.* 15, 173–177.

Mattioli, R., Costantino, P., Trovato, M., 2009. Proline accumulation in plants: not only stress. *Plant Signal. Behav.* 4, 1016–1018.

Moeletsi, M.E., 2017. Mapping of maize growing period over the Free State province of South Africa: Heat Units Approach. *Adv. Meteorol. art ID 7164068*.

Mu, X., Chen, Q., Chen, F., Yuan, L., Mi, G., 2018. Dynamic remobilization of leaf nitrogen components in relation to photosynthetic rate during grain filling in maize. *Plant Physiol. Biochem.* 129, 27–34.

Ning, P., Fritsch, F.B., Lia, C., 2017. Temporal dynamics of post-silking nitrogen fluxes and their effects on grain yield in maize under low to high nitrogen inputs. *Field Crops Res.* 204, 249–259.

Ördög, V., 1982. Apparatus for laboratory algal bioassay. *Int. Revue Gesamten Hydrobiol.* 67, 127–136.

- Póthe, P., Gergely, I., Ördög, V., 2014. Effect of microalgae leaf treatments on sunflower growth and production. Pannonian Plant Biotechnology Association Conference for PhD Students in Plant Biology, Mosonmagyaróvár, pp. 73–75.
- Prasanna, R., Bidyarani, N., Babu, S., Hossain, F., Shivay, Y.S., Nain, L., 2015. Cyanobacterial inoculation elicits plant defence response and enhanced Zn mobilization in maize hybrids. *Cogent Food Agric.* 1, 507–998.
- Shafiq, S., Akram, N.A., Ashraf, M., 2019. Assessment of physico-chemical indicators for drought tolerance in different cultivars of maize (*Zea mays* L.). *Pak. J. Bot.* 51, 1241–1247.
- Sharma, V., Prasanna, R., Hossain, F., Muthusamy, V., Nain, L., Das, S., Shivay, Y.S., Kumar, A., 2020. Priming maize seeds with cyanobacteria enhances seed vigour and plant growth in elite maize inbreds. *3 Biotech* 10, 154. <https://doi.org/10.1007/s13205-020-2141-6>.
- Singh, S., 2014. A review on possible elicitor molecules of cyanobacteria: their role in improving plant growth and providing tolerance against biotic or abiotic stress. *J. Appl. Microbiol.* 117, 1221–1244.
- Singh, J.S., Kumar, A., Rai, A.N., Singh, D.P., 2016. Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. *Front. Microbiol.* 7, 1–19.
- Stirk, W.A., Rengasamy, K.R.R., Kulkarni, M.G., van Staden, J., 2020. Plant biostimulants from seaweed: an overview. In: Geelan, D., Xu, L. (Eds.), *The Chemical Biology of Plant Biostimulants*. John Wiley and Sons Ltd, pp. 33–55.
- Szabados, L., Savouré, A., 2009. Proline: a multifunctional amino acid. *Trends Plant Sci.* 15, 89–97.
- Takács, G., Stirk, W.A., Gergely, I., Molnár, Z., van Staden, J., Ördög, V., 2019. Biostimulating effects of the cyanobacterium *Nostoc piscinale* on winter wheat in field experiments. *S. Afr. J. Bot.* 126, 99–106.
- Tóth, J., Gergely, I., Berzsenyi, Z., Ördög, V., 2019. Influence of *Nostoc entophyllum* and *Tetracystis* sp. on winter survival of rapeseed. *J. Agric. Sci. Technol. B* 9, 251–271.
- Trivedi, K., Vijay Anand, K.G., Kubavat, D., Kumar, R., Vaghela, P., Ghosh, A., 2017. Crop stage selection is vital to elicit optimal response of maize to seaweed bio-stimulant application. *J. Appl. Phycol.* 29, 2135–2144.
- Trivedi, K., Vijay Anand, K.G., Vaghela, P., Ghosh, A., 2018. Differential growth, yield and biochemical responses of maize to the exogenous application of *Kappaphycus alvarezii* seaweed extract, at grain-filling stage under normal and drought conditions. *Algal Res.* 35, 236–244.
- Vesali, F., Omid, M., Mobli, H., Kaleita, A., 2017. Feasibility of using smart phones to estimate chlorophyll content in corn plants. *Photosynthetica* 55, 603–610.
- Wang, Y., Huang, Y., Fu, W., Guo, W., Ren, N., Zhao, Y., Ye, Y., 2020. Efficient physiological and nutrient use efficiency responses of maize leaves to drought stress under different field nitrogen conditions. *Agronomy* 10, 1–14.
- Žizková, E., Kubes, M., Dobrev, P., Příbyl, P., Šimura, J., Zahajská, L., Drábková, L.Z., Novák, O., Motyka, V., 2017. Control of cytokinin and auxin homeostasis in cyanobacteria and algae. *Ann. Bot.* 119, 151–166.