Plant Growth Promoting Potential of *Phormidium* sp. ISC108 on Seed Germination, Growth Indices and Photosynthetic Efficiency of Maize (*Zea mays* L.)

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Abstract

Cyanobacteria are oxygenic, photosynthetic prokaryotes with unique potential to enhance plant growth, development, and productivity. The effects of *Phormidium* sp. ISC108 was evaluate on seed germination, seedling growth criteria and photosynthetic efficiency of maize. *Phormidium* sp. ISC108 was applied to soil via irrigation whereas control group was irrigated via sterilized water. A significant promotion in seed germination was observed in *Phormidium* sp. ISC108 treated maize seeds. Similarly, seedling growth indices of treated plants including biomass production, plant heights and leaf area significantly enhanced. Chlorophyll content as well as chlorophyll a fluorescence kinetics was also positively influenced by *Phormidium* sp. ISC108 application. Quantum yield of photosynthesis, primary photochemical reactions, rate of exiton transfer to electron transport chain, yield of electron transport and performance index of photosynthetic apparatus significantly increased in response to *Phormidium* sp. ISC108 application in soil. However, values of thermal dissipation of absorbed light showed a significant reduction as a result of *Phormidium* sp. ISC108 treatment. Considering remarkable growth promoting properties, *Phormidium* sp. ISC108 may offer a feasible economical and environmental-friendly candidate as bio-fertilizer in sustainable maize production.

Keywords: Biofertilizer; Chlorophyll a fluorescence, Cyanobacteria, *Phormidium* sp. ISC108; *Zea mays*.

Introduction

Cyanobacteria (blue green algae) are diverse group of photoautotrophic prokaryotes inhabiting different types of aquatic and terrestrial environments. Cyanobacteria can occur in nature in unicellular and filamentous, free-living and symbiotic forms (Dorador et al., 2008). They possess a unique ability to form association with different plant species and contribute to productivity in variety of agricultural and ecological situations (Karthikeyan et al., 2007). In recent years, the use of cyanobacteria in agriculture has

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received increased attention, due to their various phyto-metabolic and biological active compounds (Mohan and Kumar, 2017). Most, though not all, of non-heterocystous cyanobacteria can fix atmospheric N_2 and convert it into available form of ammonia that boosts plant growth. In addition to providing nitrogenous compounds, cyanobacteria also produce various growth promoting substances that can benefit plants. These substances can be categorized into plant hormones (e. g. gibberellins, cytokinins and auxins), vitamins (e. g. vitamin B group), amino acids and antibiotics (Sergeeva et al., 2002; Tsavkelova et al., 2006; Lu and Xu, 2015; Rai et al., 2019). Using cyanobacteria as bio-fertilizer, may offer a low cost, environment-friendly and sustainable alternative to chemical fertilizers. The role of cyanobacteria as bio-fertilizers has been mostly limited to its relevance and utilization in paddy fields of rice (Ito and Watanabe, 1985; Kyndiah and Rai, 2007; Prasanna et al., 2009; Majeed et al., 2017). Little information is available on the beneficial effects of cyanobacteria on other crops. Maize *(Zea mays* L.) is one of the most important crops, grown widely throughout world in tropical, subtropical temperate climate zones. Maize is cultivated over 170 million hectares worldwide and presently is responsible for providing approximately half of calories consumed worldwide (Liang et al., 2018). The increasing world food demand as well as cost and the contamination effect of chemical fertilizers, increase the importance bio-fertilizers such as cyanobacteria as a proper candidate in sustainable agriculture. Most reports available on beneficial effects of cyanobacteria have focused on nitrogen fixation potential and the plant growth promoting activities of non-nitrogen fixating cyanobacteria such as *Phormidium* sp. has been neglected (Mazhar et al., 2013). In the present study, we aimed to determine the possible beneficial effects of *Phormidium* sp. ISC108 as a bio-fertilizer on rate of seed germination, growth induction and photosynthetic parameters of Karoon mazie cultivar.

Materials and Methods

Plant materials and growing condition

Seeds of Karoon maize (*Zea mays* L.) genotypes were obtained from seed and plant improvement institute of Iran (SPII). After sterilization, seeds were sawn in autoclaved soil composed of 27% sand, 13% clay, 40% silt and 20% perlite. Plants were grown in 70% of soil water holding capacity (WHC) under controlled condition (25± 2°C day/ 20 ± 2°C night, 16 h photoperiod with light intensity of 120 $\mu mol.m^{-2}s^{-1}$ and 40- 50% relative humidity) for 30 days (Mohan and Kumar, 2017).

Phormidium treatment

Phormidium sp. ISC108 was obtained from the research institute of applied sciences of Shahid Beheshti University. *Phormidium* sp. ISC108 was cultured in liquid BG-11 medium (Waterbury and Stanier, 1981) and was kept in a 16/8 h light/dark photoperiod regime under 120 $\mu mol.m^{-2}s^{-1}$ light intensity at 23 ± 2°C. *Phormidium* sp. ISC108 was applied to soil via irrigation by adding 3 ml of culture (OD600: 0.7) per seed 3 times a week

(Karthikeyan et al., 2007). Control group was irrigated via sterilized water without cyanobacteria.

Seed germination analysis

Seed surfaces were sterilized, placed on filter papers in Petri dishes and soaked in *Phormidium* sp. ISC108 culture (OD600: 0.7). Seeds were placed in 25± 2°C in dark condition for 5 days. Emerged radical from seed coat was considered as criteria for seed germination. Germinated percentage (GP) was determined based on following formula:

GP (%) = (n/N).100

where n is the number of germinated seeds during the experiment and N is total number of seeds (Maguire, 1962).

Measurement of growth indices

After 30 days sawing, the shoot and root length and leaf area harvested plant was measured and analysed using Image J software (version 1.44P; US National Institutes of Health, Bethesda, Maryland, USA). Harvested plants were dried in oven (72 h at 50°C) and dry weight was determined (Rezaee et al., 2019).

Determination of chlorophyll (chl) content

Chlorophylls were extracted from 0.1 g fresh leaves with 10 ml of 80% acetone. The absorbance of supernatants was measured at 645 and 663 nm via spectrophotometer. The content of chlorophyll *a* and *b* was calculated based on following formula: chl a ($mg.g^{-1}$) = [(12.7*A663-2.69*A645) V/W], chl b ($mg.g^{-1}$) = [(22.9*A645-4.68*A663) V/W], where A= absorbance at specific wavelengths, V= final volume of chl extracts in acetone and W= fresh weight (Arnon 1949).

Measurement of chlorophyll a fluorescence transient (JIP- test) induction kinetics

Chlorophyll *a* fluorescence measurements were performed on attached leaves in room temperature using plant efficiency analyzer (Handy PEA, Hansatech instruments Ltd, Lynn, UK). Samples were dark adapted for 30 min prior to experiment. The fluorescence rise after illumination was recorded and analyzed with biolyzer 4HP software (Bioenergitic laboratory, University of Geneva, Switzerland). Definition of fluorescence kinetic parameters extracted from JIP test is presented in Table 1.

Table 1. Definition of selected parameters calculated from OJIP chlorophyll *a* fluorescent transient.

Parameter	Definition
F_V/F_M	Maximal quantum yield of PSII
φP_O	Maximal quantum yield of primary photochemical events
Ψ_O	Exiton transfer efficiency to electron transport chain
φD_O	Thermal dissipation yield
φE_O	Electron transport yield
PI	Performance index (absorbance)

Statistical analysis

The data were obtained from three independent replications and expressed as mean ± SD. The statistical significance was tested using Student's t-test. The value of P≤0.05 was used for criterion of significance. Analysis were performed using SAS software (*Statistical Analysis* System, SAS Institute Inc. Carry, NC, USA).

Results

Effects of *Phormidium* sp. ISC108 treatment on seed germination is depicted in Figure 1. Seed germination was significantly increased in presence of *Phormidium* sp. ISC108 (100% seed gaermination) compared to control group (75%).

Phormidium sp. ISC108 treatment resulted in improvement of maize growth indices (Fig. 2 and Fig. 3). Shoot and root dry weight significantly (P≤0.05) increased upon *Phormidium* sp. ISC108 treatment in soil (Fig. 2). Similarly, Root and shoot of *Phormidium* sp. ISC108 treated plant exhibited significantly higher length compared to control group (Fig. 2).

Leaf area of maize plants was also significantly (P≤0.05) affected by *Phormidium* sp. ISC108 treatment (Fig. 3). Leaf area increased approximately 2.4 fold in response to *Phormidium* application in soil.

Changes in fast fluorescene induction kinetics as result of *Phormidium* treatment are presented in Fig. 5. the maximum quantum yield (F_V/F_M), yield of primary photochemical reactions (φPO), Exiton transfer to electron transport chain (ΨO), electron transport yield (φEO) and performance index (PI) significantly (P≤0.05) increased in response of *Phormidium* application in soil (Fig. 5). However, thermal dissipation of absorbed light (φDO) significant reduced in maize plants as a result of *Phormidium* treatment (Fig. 5).

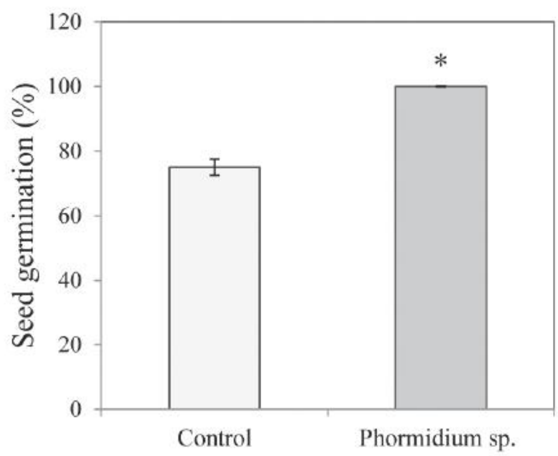

Fig. 1. Effects of *Phormidium* sp. ISC108 application on seed germination percentage of maize, 5 day after sawing. Values are the mean of three independent experiments ± standard deviation. *, different from control at p ≤ 0.05.

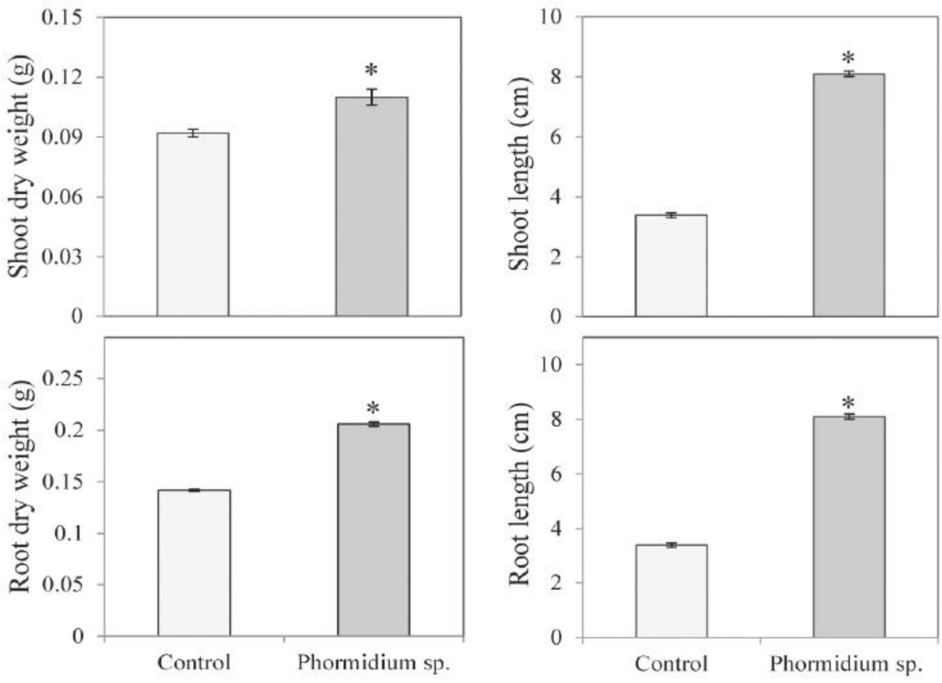

Fig. 2. Effects of *Phormidium* sp. ISC108 application on shoot and root dry weight (g) and length (cm) of maize. Values are the mean of three independent replication ± standard deviation. * different from control at $p \leq 0.05$.

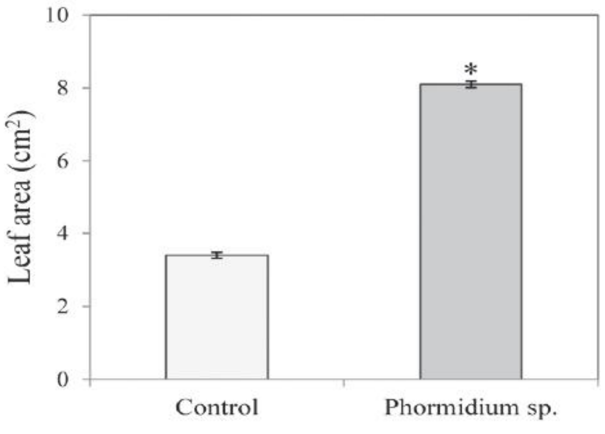

Fig. 3. Effects of *Phormidium* sp. ISC108 application on leaf area of maize. Values are the mean of three independent replication ± standard deviation. *, different from control at $p \leq 0.05$.

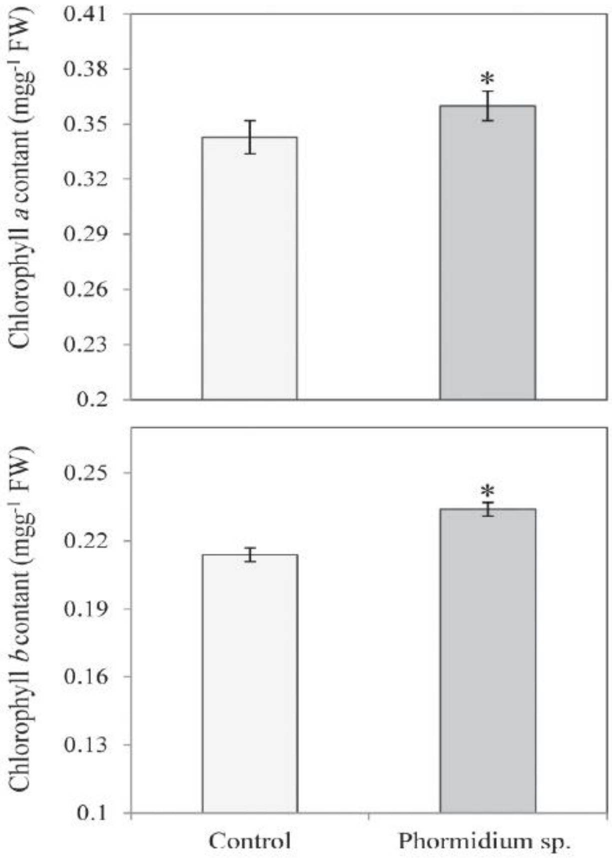

Fig. 4. Effects of *Phormidium* sp. ISC108 application on chlorophyll *a* and *b* content of maize. Values are mean of three independent replication ± standard deviation. *, different from control at $p \leq 0.05$.

Discussion

During recent years, researches on sustainable agriculture has been mainly focused on utilization of microorganisms, particularly cyanobacteria (Singh et al., 2016). It has been proven that cyanobacteria may contribute to plant growth promotion by different potential mechanisms. In the present study, the positive effects of *Phormidium* sp. ISC108 on seed germination of Karoon maize cultivar were found significant compared to control group. The obtained results are in line with other experiments indicating stimulatory effects of cyanobacteria on seed germination. The incremental effect of *Nostoc muscorum* on seed germination in several crop plants such as sorghum, wheat, lenti and sugar beet has been reported (Adam, 1999; Aly et al., 2008). Similarly, treatment with the culture filtrates of *Anabaena oryzae, Nostoc calcicola, Microchaete tenera* and *Cylindrospermum muscicola* enhanced seed germination of rice (Hassan and Morocos, 2006). Positive effects of *Anabaena variabi-*

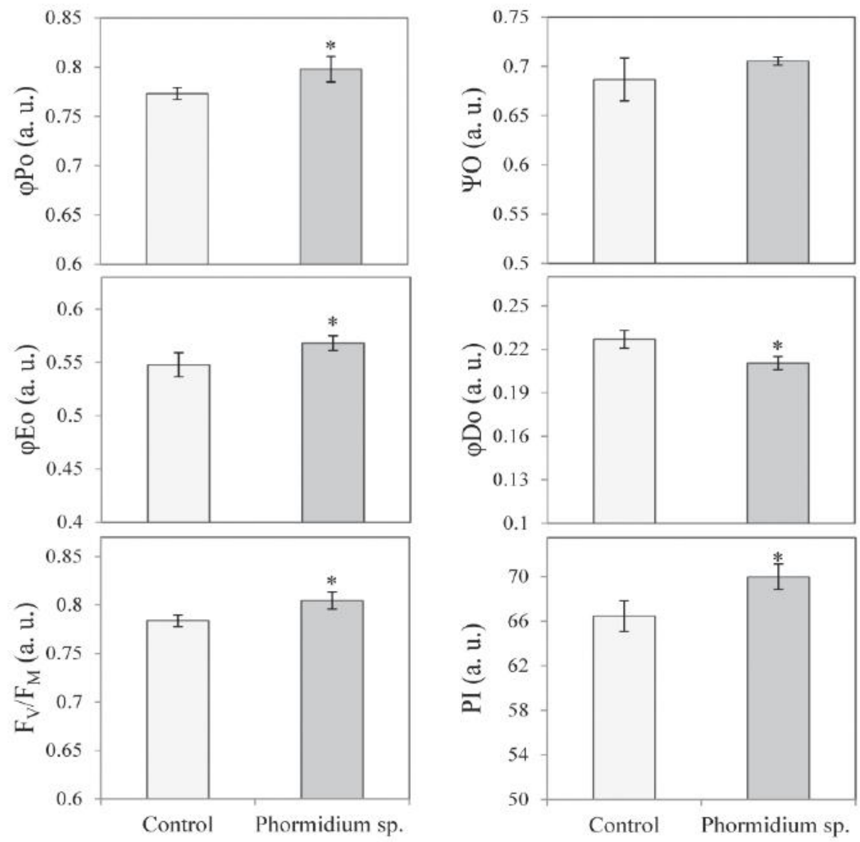

Fig. 5. Effects of *Phormidium* sp. ISC108 application on photosynthetic parameters of maize. Values are mean of three independent replication ± standard deviation. *, different from control at $p \leq 0.05$.

lis, Nostoc muscorum, Aulosira fertilissima and *Tolypothrix tenuis* on wheat seeds germination velocity and vigor has been proven (Kumar and Kaur, 2014). Consistent with our findings, Suresh et al. (2019) observed 100% seed germination percentage of *Zea mays* and *Sorghum bicolour* treatment with 200 and 500 $mg.g^{-1}$ growth promoting substances of *Anabaena variabilis* compared to control group (25% and 45% for control group of *Zea mays* and Sorghum bicolour, respectively).

Application of *Phormidium* sp. ISC108 as bio-fertilizer greatly enhanced plant growth parameters of Karoon maize cultivar. Our data revealed a significant difference in biomass production, plant heights as well as leaf area between *Phormidium* sp. ISC108 treated plants and control group. In accordance with our results, Shariatmadari et al. (2013) reported a significant promotion of growth and morphological parameters of squash, cucumber, and tomato plants treated with *Anabaena vaginicola* and *Nostoc calcicola*. Moreover, research conducted with Hussain and Hasnain (2011) and Mazhar et al. (2013) revealed the improvement of shoot length, lateral root fromation, dry weight as well as leaf area of whaet plants treated with several cyanobacterial strains including *Nostoc* sp. *Anabaena* sp. *Chroococcidiopsis* sp., *Calothrix* sp. and *Phormidium* sp. Apparantly, positive impact of cyanobacterial application on seed germination and seedling growth indices can be contributed to the wide array of bio-active compounds produced by cyanobacteria which could either directly or indirectly be involved in cell division, cell expansion and root initaiation (Prasanna et al., 2010; Shariatmadari et al., 2013). It has been demonstrated that *Phormidium* sp. is cpable of synthesis almost all clasess of phytohormones, including Auxins, cytokinins and gibbrelins. These phytohormones may be involved in induction of biomass production, boost growth and development, as well as increase seed germination and viability (Rai et al., 2019).

Photosynthesis is one of the most important biological process that is particularly influenced by external factors. The grwoth and yield of plants are ultimately determined by the photosynthetic capacity. Chlorophyll molecules play a crucial role in light dependent photosynthetic reactions by capturing light energy and transfering absorbed energy to photosystems reactions centers (Ghotbi-Ravandi et al., 2019). In this study, chlorophyll content of maize leaves responded positively to *Phormidium* sp. ISC108 inoculation. Content of both chlorophyll a and b significantly increased in *Phormidium* sp. ISC108 treated plants compared to control group. Our results corroborated the work of Grzesik and Romanovska-Duda (2015) which reported an increase in chlorophyll index of willow plants treated with *Microcystis aeruginosa* and *Anabaena* sp. Increase in chlorophyll content might be due to higher availabilty of microelemnts necessary for chlorophyll biosynthesis pathway, such as iron and magnesium suplemented by cyanobacteria (Obana et al., 2007).

In the present study, poly-phasic chlorophyll a fluorescence technique was used to determine the effects of *Phormidium* sp. ISC108 application on photosynthetic efficiency of maize. Chlorophyll a fluorescence parameters provide a reliable method to probe physiological and biochemical state of photosynthetic light reactions (Kalaji et al., 2012). Maximum quantum yield of PSII (F_V/F_M) is one of the most sensitive parameters of photosynthesis. Quantum yield represents the moles of CO_2 fixed per quanta (photons) absorbed. On the other hand, it depicts the efficiency of light energy conversion into chemical carbon bonds (Ghotbi-Ravandi et al., 2014). Under controlled condition, the value of F_V/F_M is near 0.8-0.83 in most plant (Bjorkman and Demming, 1987). In the present study, values of F_V/F_M significantly increased in response to *Phormidium* sp. ISC108 treatment. In addition to quantum yield, yield of primary photochemistry (φPO) was also increased significanly in *Phormidium* sp. ISC108 treated plants. Higher chlorophyll content and reduction in dissipation of absorbed light through heat or fluorescence result in higher energy flow to reaction centers (RCs) and increase in F_V/F_M and φPO values (Ghotbi-Ravandi et al., 2016). Significant reduction in thermal dissipation yield (φDO) in *Phormid-*

ium sp. ISC108 treated plants is consistent with increase in quantum yield and primary photochamical events. Higher rate of P680 excitation results in higher electron transfer to secondary electron acceptor (ΨO) and downstream into electron transfer chain (φEO). In our experiments, *Phormidium* sp. ISC108 treated plant exhebited an increase in both ΨO and φEO vlues. Performance index (PI) is one of the most important parametrs of chlorophyll a fluorescence which depicts the overal effficiency of photosynthtic light reactions. PI is a combination of number of active RCs, energy flux to photosystem II and yield of electron trasport (Zushi et al., 2012). In the present study, treatment of mazie with *Phormidium* significantly increased the value of PI. Higher values of PI in can be contributed to overal higher photosynthetic efficiency in *Phormidium* sp. ISC108 treated maize plants.

In our experiments, the beneficial effects of *Phormidium* sp. ISC108 in term of seed germination, growth parameters and photosynthetic capacity of Karoon maize cultivar were found to be significant. *Phormidium* sp. ISC108 can be considered as valuabe candidate for formulation of low cost and effective biofertilzer for sustanable maize production.

References

Adam MS. (1999). The promotive effect of the cyanobacterium *Nostoc muscorum* on the growth of some crop plants. Microbiologica polinica. 48: 163-171.

Aly MHA, Abd El-All Azza AM, Mostafa SS. (2008). Enhancement of sugar beet seed germination, plant growth, performance and biochemical compounds as contributed by algal extracellular products. Journal of Agricultural Sciecne, Mansoura University. 33 (12): 8429-8448.

Arnon DI. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiology. 24: 1-15.

Bjorkman O and Demmig B. (1987). Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta. 170 (4): 489-504.

Dorador C, Vila I, Imhoff JF, Witzel KP. (2008). Cyanobacterial diversity in Salar de Huasco, a high altitude saline wetland in northern Chile: an example of geographical dispersion? FEMS Microbiology Ecology. 64 (3): 419-432.

Ghotbi-Ravandi AA, Shahbazi M, Pessarakli M, Shariati M. (2016). Monitoring the photosystem II behavior of wild and cultivated barley in response to progressive water stress and rehydration using OJIP chlorophyll a fluorescence transient. Journal of Plant Nutrition. 39 (8): 1174-1185.

Ghotbi-Ravandi AA, Shahbazi M, Shariati M, Mulo P. (2014). Effects of mild and severe drought stress on photosynthetic efficiency in tolerant and susceptible barley (*Hordeum vulgare* L.) genotypes. Journal of Agronomy and Crop Science. 200 (6): 403-415.

Ghotbi-Ravandi AA, Shariati M, Shahbazi M, Shobbar ZS. (2019). Expression pattern and physiological roles of plastid terminal oxidase (PTOX) in wild and cultivated barley genotypes under drought stress. Environmental and Experimental Botany. 162: 319-320.

Grzesik M and Romanovska-Duda Z. (2015). Ability of cyanobacteria and green algae to improve metabolic activity and developmentof willow plants. Polish Journal of Environmental Studies. 24 (3): 1003-1012.

Hassan EA and Morcos MM. (2006). Bioenhancement effect of cyanobacteria on rice seeds germination and seedlings growth. Journal of Agricultural Sciecne Mansoura University. 31 (8): 5351-5361.

Hussain A and Hasnain S. (2011). Phytostimulation and biofertilization in wheat by cyanobacteria. Journal of Industrial Microbiology and Biotechnology. 38 (1): 85-92.

Ito O and Watanabe I. (1985). Availability to rice plants of nitrogen fixed by *Azolla*. Soil Science and Plant Nutrition. 31 (1): 91-104.

Kalaji HM, Carpentier R, Allakhverdiev SI, Bosa K. (2012). Fluorescence parameters as early indicators of light stress in barley. Journal of Photochemistry and Photobiology B: Biology. 112 (2): 1-6

Karthikeyan N, Prasanna R, Nain L, Kaushik BD. (2007). Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. European Journal of Soil Biology. 43 (1): 23-30.

Kumar A and Kaur R. (2014). Impact of cyanobacterial filtrate on seed germination behaviour of wheat. International Journal of Basic and Applied Biology. 1 (1): 11-15.

Kyndiah O and Rai AN. (2007). Induction of sporulation by sulphate limitation in *Nostoc* ANTH, a symbiotic strain capable of colonizing roots of rice plants. Indian Journal of Biotechnology. 6: 57-62.

Liang Z, Pandey P, Stoerger V, Xu Y, Qiu Y, Ge Y and Schnable JC. (2018). Conventional and hyperspectral time-series imaging of maize lines widely used in field trials. Giga Science. 72 (2): 1-11.

Lu Y and Xu J. (2015). Phytohormones in microalgae: a new opportunity for microalgal biotechnology? Trends Plant Science. 20 (5): 273-282.

Maguire JD. (1962). Speed of germination aid in selection and evaluation for seedling emergence and vigor. Crop Science. 2: 176-177.

Majeed A, Muhammad Z, Islam S, Ullah Z, Ullah R. (2017). Cyanobacterial application as bio-fertilizers in rice fields: role in growth promotion and crop productivity. PSM Microbiology. 2: 47-50.

Mazhar S, Cohen JD, Hasnain S. (2013). Auxin producing non-heterocystoue cyanobacteria and their impact on the growth and endogenous auxin homeostasis of wheat. Journal of Basic Microbiology. 53 (12): 996-1003.

Mohan A and Kumar B. (2017). Growth performance and yield potential of cereal crops (wheat, maize and barley) in association with cyanobacteria. International Journal of Current Microbiology and Applied Science. 6 (10): 744-758.

Obana S, Miyamoto K, Morita S, Ohmori M, Inubushi K. (2007). Effect of *Nostoc* sp. on soil characteristics, plant growth and nutrient uptake. Journal of Applied Phycology. 19 (6): 641-646.

Prasanna R, Joshi M, Rana A, Nain L. (2010). Modulation of IAA production by tryptophan and light. Polish Journal of Microbiology. 59: 99-105.

Prasanna R, Nain L, Ancha R, Shrikrishna J, Joshi M, Kaushik BD. (2009). Rhizosphere dynamics of inoculated cyanobacteria and their growth promoting role in rice crop. Egyptian Journal of Biology. 11: 26-36.

Rai AN, Singh AK, Syiem MB. (2019). Plant growth promoting Abilities in Cyanobacteria. In: Mishra AK, Tiwari DN, Rai AN (eds) Cyanobacteria, Academic Press. 459-476.

Rezaee M, Ghotbi Ravandi AA, Hassani SB, Soltani N. (2019). *Phormidium* improves seed germination and growth parameters of *Trifolium alexandrinum* in hexadecane-contaminated soil. Journal of Phycological Research. 3 (1): 312-325.

Sergeeva E, Liaimer A, Bergman B. (2002). Evidence for production of the phytohormone indole-3-acetic acid by cyanobacteria. Planta. 215 (2): 229-238.

Shariatmadari Z, Riahi H, Hastroudi MS, Ghassempour A, Aghashariatmadary Z. (2013). Plant growth promoting cyanobacteria and their distribution in terrestrial habitats of Iran. Soil Science and Plant Nutrition. 59 (4): 535-547.

Singh JS, Arun K, Rai AN, Singh DP. (2016). Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. Frontiers in Microbiology. 7: 1-19.

Suresh A, Soundararajan S, Elavarasi S, Oscar LF, Thajuddin N. (2019). Evaluation and characterization of the plant growth promoting potentials of two heterocystous cyanobacteria for improving food grains growth. Biocatalysis and Agricultural Biotechnology. 17: 476-652.

Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI. (2006). Hormones and hormone-like substances of microorganisms: a review. Applied Biochemistry and Microbiology. 42 (3): 229-235.

Waterbury JB and Stanier RY. (1981). Isolation and growth of cyanobacteria from marine and hypersaline environments. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG. (Eds.). The Prokaryotes. Vol. 1. Springer-Verlag, Berlin. Pp. 221-223.

Zushi K, Kajiwara S, Matsuzoe N. (2012). Chlorophyll a fluorescence OJIP transient as a tool to characterize and evaluate response to heat and chilling stress in tomato leaf and fruit. Scientia Horticulturae. 148: 39-46.