Phormidium sp. Improves Growth, Auxin Content and Nutritional Value of Wild Barley (*Hordeum spantaneum* L.)

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Abstract

Cyanobacteria are oxygenic, photosynthetic prokaryotes with unique potential to enhance plant growth, development, and productivity. These microorganisms have the ability to stimulate plant growth by producing growth-inducing phytohormones and increasing the solubility of soil nutrients. In the present study, the plant growth promoting potential of *Phormidium* sp. on the growth indices of wild barley seedlings was evaluated. In this regard, *Phormidium* sp. is a filamentous and non-heterocystous cyanobacterium. Indeed, filaments are unbranched and usually in fine, smooth, layered microscopic or macroscopic mats. The results showed that dry weight and length of root and shoot, the content of nitrogen (2%), potassium (2%), and auxin (8%) phytohormone of wild barley seedlings treated with *Phormidium* sp. had a significant increase compared to the control group. Accordingly, the use of *Phormidium* sp. as a plant growth-promoting cyanobacteria seems promising alternative to chemical fertilizers.

Keywords: Auxin, Biofertilizer, Cyanobacteria, Growth phytohormones, Nitrogen content

Introduction

The production of healthy plant-based products to meet the nutritional needs of the world's growing population depends on the use of fertilizers to supply plant nutrients. The use of microorganisms as bio-inoculants is believed to be echo-friendly approach to maintain soil fertility (Kour et al., 2020). Some microorganisms especially plant growth-promoting (PGP) bacteriacan enhance plant growth and protect plants from disease and abiotic stresses (Souza et al., 2015). Micro-organisms that are well studied for their beneficial effects are the plant growth-promoting rhizobacteria (PGPR), mycorrhizal fungi and symbiotic rhizobia (Castro-Sowinski et al., 2007; Yang et al., 2009; Wang et al., 2012; Mendes et al., 2013; Willis et al., 2013). Along with the fungal and bacterial species, cyanobacteria are another group of microorganisms that potentially improve soil fertility and crop productivity through contribution in

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biological nitrogen fixation, phosphate solubilization, mineral release, phytohormone secretion and siderophore production (Hu et al., 2002; Singh, 2014). Cyanobacteria distributed in a wide range of harsh habitats including fresh water, hot springs, arctic and antarctic (Singh, 2014). *Phormidium* sp. is a genus of filamentous cyanobacteria and belongs to Oscillatoriales order (cheng et al., 2019). *Phormidium* sp. has the ability to produce cytokinin (Hussain et al., 2010), auxin (Boopathi, 2013), and gibberline (Gupta and Agarwal, 1973) hormones. It is observed the seed germination of tobacco increased up to 40% by adding the extracellular extract (ECE) of this cyanobacterium to tobacco culture medium (Boopathi, 2013). Also, it was found that *Phormidium* was the superior species for biological crust formation (Hu et al., 2002). This cyanobacterium has received less attention as a plant growth-promoting bacteria.

Hordeum vulgare L. subsp. *Spontaneum* is considered as progenitor of cultivated barley (Ghahremaninejad et al., 2021) and its hybrid with cultivated barley is fertile. This plant is a source of various stress-resistant genes and used as a model for modification of cultivated barley (Guo et al., 2009). The aim of this study was to investigate the potential of *Phormidium* sp. to elicit the growth of wild barley and possible underlying mechanisms.

Material and methods

Plant Material and Growth Condition

The seeds of wild barley (HS) were obtained from "Seed and Plant Research Improvement Institute". The barley seeds were surface sterilized in 70% ethanol for about 2 min, 6% sodium hypochlorite solution for about 5min, rinsed three times in distilled water, and then placed on wet filter paper in Petri dishes to germinate in 25 °C. Later, germinated seeds were sown in pots containing 30% perlite and 70% peat moss and grown under controlled condition after 30 hours later, (25 °C, 16 h photoperiod with light intensity of 74 µmol photons $m^{-2}s^{-1}$ and 65% relative humidity). After seeds sowing, the Hoagland solution was added to pots (Hoagland and Aron, 1950). Sampling from control and cyanobacterium treated plants were performed on tenth day after cyanobacterial treatment. Leafs and root samples were taken per treatment and immediately stored at -80 °C for further analysis.

Culture and purification of cyanobacteria

Phormidium sp. was isolated from the surface soil of an estuary in Abadan city with length 48° 18' 19,929", width 30° 27' 04, 148 and height -1.1 above sea level. The microalgae species purified through repeated subculturing in the BG11 solid medium, using artificial illumination with a 16/8 hour light-dark cycle, and 25 ± 2 °C temperature for culturing the sample. Finally, the purified cyanobacterium was identified by binocular optical microscope Olympus, Model BH-2 and based on Desikachary (1959). In this experiment, two concentration of suspensions were prepared. About two weeks before planting, 100 ml of cyanobacterial suspension with a concentration of 4 $g.L^{-1}$

was added to pots. After planting, 50 ml of cyanobacterial suspension with a concentration of 2 $g.L^{-1}$ was added to the plants.

Measurement of growth indices

Plants were grown in the greenhouse with the standard condition for ten days. After harvesting (ten day after planting), root and shoot length were measured. After that, plants were dried in oven (24 h at 50 °C) and dry weight was determined (Rezaee et al., 2019).

Measurement of auxin

The plant samples were pulverized in liquid nitrogen. In order to extract the auxin, the samples placed overnight at 4 °C in methanol with sodium diethyl dithiocarbamate. After centrifuge at 10000 g, the supernatant was removed and the residue was mixed again for 60 minutes. Next, the supernatant was vacuumed at 40 °C to remove methanol. Then, the mixture was dissolved in 2 ml of 1 M formic acid. Finally, the auxin content of samples were measured by HPLC (RI-DIV-D made in Germany) (Ge et al., 2007).

Measurement of nutrients content

In order to measure elements concentration, the roots and shoots of the plant were separated and digested with HNO_3-$HCLO_4$ (2: 1v/v). Then, determination of minerals were measured with Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (5100, Agilent, California, United States) (Guo et al., 2007).

Statistical analysis

The experiment used a completely randomized design with three replicates. Mean comparison between control and cyanobacteria treatments was performed using Student's t-test ($P \leq 0.05$).

Results

The results of the pot studies showed a significant increase in the vegetative characteristics of the treated plants compared with the controls. Additionally, *Phormidium* sp. treatment led to significant ($P \leq 0.05$) increase in shoot and root growth indicators like root length and dry weight of root and shoot. Root length (20%) and dry weight of root (10%), shoot length (30%), and dry weight of shoot (20%) enhanced by *Phormidium* sp. treatment (Fig. 1a, b).

Phormidium sp. treatment resulted in significant ($P \leq 0.05$) increase in nitrogen content in both shoot and root compared to the control group (Table 1). Potassium content in both root and shoot of wild barley was significantly increased ($P \leq 0.05$) in *Phormidium* sp. treated plants compared to the control group (Table 1). Sulfur content in plants treated with cyanobacteria decreased significantly ($P \leq 0.05$) in both shoot and root compared to the control group (Table 1). *Phormidium* sp. treatment led to significant reduction ($P \leq 0.05$) of calcium content in roots and shoots of wild barley (Table 1). The results of hormone analysis revealed a significant increase ($P \leq 0.05$) in auxin phytohormone content in *Phormidium* sp. treated seedlings compared to the control group (Figure 2).

Discussion

Cyanobacteria and microalgae are one of

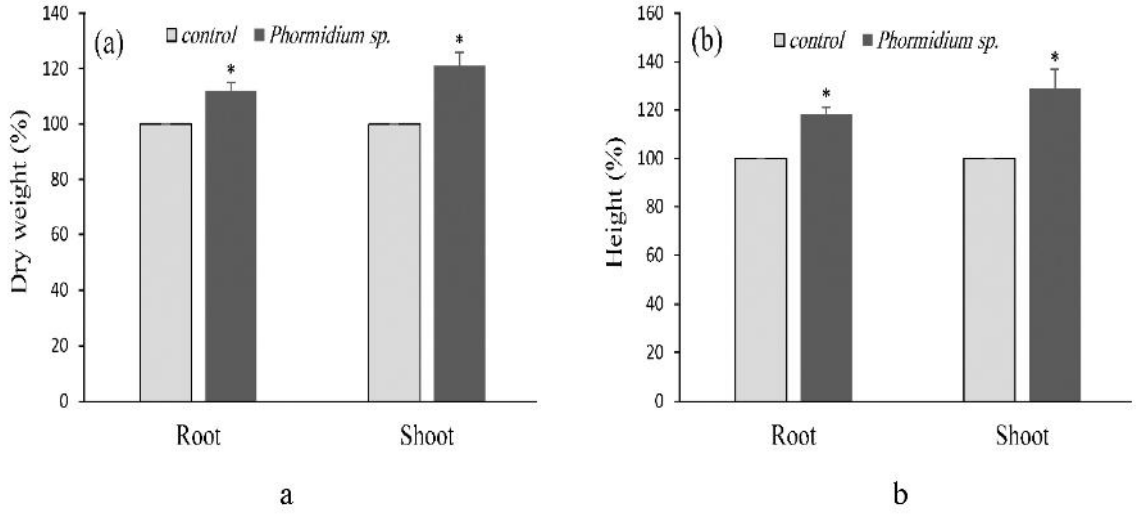

Fig. 1. Effects of *Phormidium* sp. treatment: (a) on dry weight (%), and (b) height (%) of root and shoot in wild barley *(Hordeum spantaneum*)

Table 1. Effects of *Phormidum* sp. treatment on nutrient content in root and shoot of wild barley (*Hordeum spantaneum*)

		Nutrient content (µg g^{-1})			
Organ	Treatment	N	K	S	Ca
Root	Control	3225.9 ± 22.8	2481.7 ± 24.2	187.6 ± 12.0	1076.7 ± 12.5
	Phormidium sp.	3369.6 ± 86.6 *	2570.2 ± 21.8 *	172.9 ± 22.8 *	1038.3 ± 22.7 *
Shoot	Control	8811.2 ± 89.7	3474.5 ± 33.9	102.2 ± 11.3	1040.6 ± 5.1
	Phormidium sp.	8995.8 ± 76.4 *	3598.3 ± 30.5 *	81.2 ± 16.8 *	1021.8 ± 6.5 *

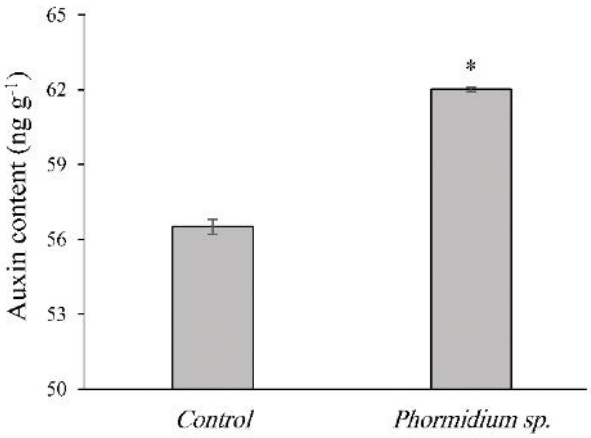

Fig. 2. Effects of *Phormidium* sp. treatment on shoot auxin content (ng.g^{-1}) of wild barley (*Hordeum spantaneum*)

the primary photosynthetic microorganisms of the soil. Due to the important characteristic of nitrogen fixation, the utility of cyanobacteria in agriculture to enhance production is beyond doubt (Singh, 2014). In the present study, it found that the use of *Phormidium* sp. promoted the growth indices of wild barley. *Phormidium* sp. increased the dry weight and length of roots and shoots of wild barley. Similar to our findings, it reported that *Phormidium* significantly increased dry weight and length of roots and shoots in *Zea mays* (Younesi et al., 2019). This enhancement in growth and dry matter production may be due to the secretion of phytohormones such as auxin (Boopathi et al., 2013), gibberellin (Gupta and Agarwal, 1973), and zeatin (Hussain et al., 2010; Hussain and Hasnain, 2011) by *Phormidium* sp. Indeed, auxin (Indole-3-acetic acid, IAA) content in the shoot of wild barley increased by *Phormidium* sp. Additionally, auxin is a key regulator of plant growth and development, orchestrating cell division, elongating and differentiating, developing embryo, tropismming root and stem, apical dominance, and transiting to flowering (Balzan et al., 2014). Recently IAA phytohormone isolated from *Phormidium* sp. that coexist with mangrove root (Boopathi et al., 2013). Also, IAA was isolated from *Phormidium* sp. in a study by Hossein et al. (2010).

In the present study, nitrogen and potassium content of both shoot and root tissues were significantly enhanced by *Phormidium* sp. Treatment. While, *Phormidium* is a non-heterocystous cyanobacterium, that can stabilize nitrogen in the absence of oxygen or micro-oxyc condition (Bergman et al., 1997). Similar to our findings, it was reported that *Phormidium ambiguum*, as a non-stabilizing nitrogen cyanobacterium, was able to increase soil nitrogen levels (Chamizo et al., 2018). Indeed, cyanobacteria play an important role in the bio-nutrient cycle. Additionally, they have an extraordinary ability to manage agricultural ecosystems. Further, they improve potassium, iron and other soil nutrients and facilitate the use of these nutrients for plants (Singh, 2015). In the present study, potassium content of both root and shoot of wild barley significantly increased in response to *Phormidium* sp. treatment. Similar to our results, an increase in potassium and phosphorus content observed in tomato seedlings treated with *Aphanothece* sp. cyanobacterium (Mutale-joan et al., 2020). In this experiment, cyanobacterial treatment reduced the amount of sulfur in both root and leaf tissues of wild barley. The reduction in plant sulfur content may be due to the consumption of part of soil sulfur by cyanobacteria. In this regard, iron-sulfur clusters (Fe-S) act as a protein cofactor in many important physiological processes including photosynthesis, respiration, and nitrogen fixation of cyanobacteria and other photosynthetic organisms (Balk and Pilon, 2011). In the study performed by Aziz and Hashem (2003), cyanobacterial inoculation slightly increased the available sulfur content of the soil compared to the control. So far, not much research has been done on the effect of cyanobacteria on the solubility of sulfur

and potassium in the soil and its uptake by plants.

Furthermore, the calcium content of both root and shoot was also significantly decreased in response to *Phormidium* sp. treatment. Since *Phormidium* has the ability to calcify, calcium in the soil may be used in the calcification process (Shiraishi et al., 2017). In the chamomile plant treated by *Nostoc carneum* ISB88, *Nostoc punctiforme* ISB90, and *Wollea vaginicolla* ISB89 it was found that the amount of soil calcium has also increased (Zarezadeh et al., 2020). As yet, very little research has been done on the distribution of calcium and sulfur in the soil by cyanobacteria.

In general, according to the results of this experiment, *Phormidium* sp. enhanced the content of nitrogen, potassium elements and auxin phytohormone. As a result, plant growth indices such as dry weight, root and shoot length have increased. Therefore, the use of this cyanobacterium as a plant growth promoting rhizobacteria (PGPR) seems promising ecofriendly method in boosting growth of barley plants.

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