Effect of PGPR *Arthrobacter* sp. CTF1 and foliar iodine spraying on pea microgreens growth in hydroponic culture

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Abstract. The use of plant growth-promoting rhizobacteria (PGPR) along with additional biofortification of agricultural plants with scarce essential elements, such as iodine, is a promising area of agricultural biotechnology. The seeds of *Pisum sativum* L. (var. Madras) pre-inoculated for two hours with PGPR *Arthrobacter* sp. strain CTF1 (10^8 CFU/mL) were grown for 14 days in a hydroponic culture at foliar spraying with iodine solution (0.01% KI or KIO_3) on the 7th day of the vegetation. Growth parameters such as the length of shoot, fresh and dry biomass of seedlings were studied, the germination percentage and vigor index were calculated, and the content of photosynthetic pigments in pea leaves was assessed. The results showed inoculation of pea seeds with PGPR strain CTF1 had a positive effect on the biomass of two-week-old pea microgreens and their vigor index. At the same time, a significant increase in photosynthetic pigments was also observed in the leaves of pea seedlings, especially chlorophyll *a* (by almost 25%) and carotenoids (by almost 40%). Additionally, application of iodine via foliar spraying, irrespective of its form (KI or KIO_3), resulted in nearly a 26-fold surge in amount of microgreens. However, the significant effect of such iodine treatment had a positive effect only on the content of carotenoids.

1 Introduction

Sustained population growth is accompanied by increasing demand for food, making nutritional security a critical challenge for many countries [1–3]. Over the past decades, the scale of agricultural production has increased due to increased application of agrochemicals, which creates risks for both human health and the environment [4]. The use of biofertilizers based on plant growth-promoting rhizobacteria (PGPR) is a promising area of agricultural biotechnology [5]. PGP-rhizobacteria are capable of fixing atmospheric nitrogen, solubilizing compounds of phosphorus, potassium, iron, zinc, etc. that are unavailable to plants, as well as producing phytohormones and siderophores, which helps to increase plant

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productivity and their resistance to stress [5–7]. Moreover, inoculation with PGPR is one of the ways to biofortify crops, including microgreens, with macro- and microelements and biologically active substances, which will reduce global malnutrition [6, 8].

Human well-being requires at least 22 mineral elements, one of which is iodine [3]. Iodine (I) take part in important metabolism processes. Iodine deficiency will cause inadequate synthesis of thyroid hormone that has multiple functions such as enhancing protein synthesis, regulating energy transfer, accelerating growth and development, and maintaining central nervous system [9, 10]. Iodine is not essential for plants but may be considered to be beneficial, due to its antioxidant properties this element is able to protect plants from different forms of oxidant stress and at certain concentrations may serve as growth stimulator [11, 12].

Biofortification with iodine can be easily used in hydroponic systems for growing microgreens [11]. The effects of treating plants with iodine have been studied by many researchers [9–14], however there is still no unambiguous data on the mechanisms of its action on growth and accumulation in microgreen.

The aim of the study was to evaluate the effect of PGPR *Arthrobacter* sp. strain CTF1 on pea seedlings growth parameters in hydroponic culture and the content of photosynthetic pigments in pea microgreens after iodine spraying.

2 Materials and methods

2.1 Materials

A bacterial strain CTF1 was isolated at the end of May, 2023 from the rhizosphere of flowering plants *Tussilago farfara* L. (Asteraceae family) growing on the disturbed territory closed to copper smelter (JSC "Karabashmed", Karabash, Chelyabinsk region, Russia) and was previously identified as *Arthrobacter* sp. with 16S rRNA (rDNA) genome sequencing according to the method described previously [7]. Before testing its effect on plant growth, its resistance to iodine and antibiotics as well as PGP attributes such as indol-3-acetic acid (IAA) production, insoluble form of phosphate and zinc solubilization, siderophore and ammonia production were investigated.

Pea microgreen (*Pisum sativum* L. variety Madras) was selected to conduct the present experiment. Mature seeds, similar in size and shape, were surface sterilized (70% ethanol for 30 sec, then 4% sodium hypochlorite for 2 min) and finally washed repeatedly with sterile Millipore water (Millipore, USA). The seeds were soaked overnight and inoculated for 2-h with *Arthrobacter* sp. strain CTF1 cultures (10^8 CFU/mL), pre-grown in LB (Luria–Bertani) medium, while in the control it was only sterile LB medium. Each treatment consisted of 10 Petri dishes containing a total of 36 pea seeds, lined with moistened sterile filter paper at the bottom, and seed germination was checked daily until full sprouting. On the fifth day of the experiment, the seedlings were transplanted into plastic sprouters ("The home AeroGARDEN", SmartGidroCompany, LLC, Russia) with a hydroponic nutrient solution (calcium nitrate: 0.868 g/L; potassium nitrate: 0.426 g/L; magnesium sulfate: 0.378 g/L; monopotassium phosphate: 0.284 g/L; ferrous sulfate: 0.02 g/L; ammonium sulfate: 0.01 g/L; borax: 0.01 g/L; manganese sulfate: 5 mg/L, zinc sulfate: 0.5 mg/L; copper sulfate: 0.5 mg/L) under the following controlled environmental conditions: photosynthetic photon flux density of 180 ± 20 μmol/m^2 sec provided by phytolamps (ULI-P10-18W/SPFR IP40) with day:night regime of 14:10 at 23 ± 3 °C and grown for 14 days. Every treatment included 3 independent boxes with 40 pea seedlings in each. Upon the emergence of the first leaves (on the 7th day of growth) the seedlings were sprayed with 0.01% KI (SI1) or KIO_3 (SI2) or just with sterile water without iodine (NS).

2.2 Methods

2.2.1 Iodine test

The iodine test was performed to understand the strain resistance level. The iodine rich LB agar plates were prepared, and isolate CTF1 was subjected to an increasing concentration of two forms of iodine (KI or KIO_3): 0%, 0.001%, 0.01%, 0.1%. Following incubation at 27 °C for 3 days, colonies displaying unrestricted growth on both KI-LB and KIO_3-LB agar plates were identified as resistant to the corresponding concentration of iodine.

2.2.2 Antibiotic test

Antibiotic resistance of *Arthrobacter* sp. strain CTF1 was checked by disc diffusion method [15]. The concentrations of the antibiotic discs used were 10 µg erythromycin, 30 µg kanamycin, 30 µg streptomycin, 30 µg tetracycline, 10 µg ampicillin, 30 µg chloramphenicol, and 6 µg penicillin. The zone of inhibition around the disc was measured and categorized under as resistant, intermediate, and susceptible on the basis of company guidelines (NICF, Russia).

2.2.3 PGP features

The bacterial isolate CTF1 was subjected on MSM agar [16] supplemented with 0.1% of ZnO for 2 days at 28 °C to check their ZnO-solubilizing property. The halo zone around the bacterial colony confirms the Zn solubilizing property. The Zn solubility ratio was calculated as diameter of solubilized halo zone (i.e. halo + colony) to the diameter of the colony [17].

Other PGP features such as IAA production, phosphate solubilization, siderophore, and ammonia production were tested as described previously [18]. The IAA production was checked by appearance of pink color after adding Salkowski's reagent to freshly prepared bacterial cultures (10^8 CFU/mL) and measured at 530 nm using UV-Vis spectrophotometer (Infinite 200 PRO, Tecan, Austria). Commercially available IAA (Sigma-Aldrich) was used to prepare a calibration curve. Phosphate solubilization was confirmed by appearance of yellow color after reacting of freshly grown (10^8 CFU/mL) bacteria with vanado-molybdic reagent and measured spectrophotometrically at 420 nm. The ability to solubilize phosphates was assessed by preparing a calibration curve based on the standard solution of potassium dihydrogen orthophosphate (KH_2PO_4) and expressed in mg PO_4^{3-}/L. The ammonia production was determined by the color change from yellow to reddish-brown after incubation of the freshly prepared bacterial inoculum (10^8 CFU/mL) with Nessler's reagent.

2.2.4 Germination parameters

Germination characteristics were calculated according to Kumar et al. [19]. Germination percentage index (GPI) was calculated using the equation (1):

$$\text{GPI (\%)} = \text{total number of seeds germinated / total number of seeds} \times 100\% \quad (1)$$

Seedling vigor index (VI) was calculated using the equation (2):

$$\text{VI} = \text{germination percentage (\%)} \times \text{mean seedling dry biomass (g)} \quad (2)$$

The length of pea seedlings (only aboveground part) was measured on the 7th and 14th days of the experiment. Pea seedlings fresh biomass (FW) and dry biomass (DW) were measured at the end of the experiment (14th day) using standard laboratory equipment.

2.2.5 Photosynthetic pigment content

Photosynthetic pigments such as chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car) were extracted in 80% cold acetone, measured at 470, 647, and 663 nm and finally calculated as milligram per one gram of DW according to Lichtenthaler [20].

2.2.6 Iodine determination

The iodine content in dry plant samples was determined using a titrimetric method based on the oxidation reaction of thiocyanate ion with a mixture of nitrite and nitrate ions, catalyzed by iodide ions and finally calculated as milligram per one gram of DW [21].

2.2.7 Statistical analysis

The results were presented as mean values (Means) with standard errors (SE). The normality and homogeneity of variances were verified using the Shapiro-Wilk's and the Levene's test, respectively and significant difference between treatments were determined by analysis of variance (ANOVA) followed by Tukey's test. Different small and capital alphabetical letters indicate significant difference between treatments at $p < 0.05$.

3 Results and discussion

The bacterial culture, namely CTF1, isolated from the rhizosphere of *T. farfara*, was assessed for its morphological features (Table 1). The Gram staining test showed that strain CTF1 was positive. In addition, this strain showed fast growing properties which are very useful for conducting the experiment. The isolate was identified with 16S rRNA sequencing as *Arthrobacter* sp. with 99.47% similarity (Table 2).

Table 1. Morphological characteristics of bacterial strain CTF1 on solid LB medium.

Bacterial strain	Shape	Margin	Elevation	Texture	Pigmentation	Size	Growth
CTF1	Circular	Entire	Raised	Smooth	Cream	Large	Fast

The iodine test with KI and KIO_3 did not exhibit adverse effect on the growth of studied strain that's make it suitable for plant microgreen biofortification.

Further, the *Arthrobacter* sp. CTF1 studied strain was tested for PGP attributes. It was shown that this strain was able to synthesize IAA, solubilize insoluble phosphates and zinc compounds, as well as produce siderophores (Table 2). The ability of different *Arthrobacter* strains to exhibit various PGP-properties (synthesis of IAA, producing siderophores etc.) was also noted by other authors [22–24].

Moreover, *Arthrobacter* sp. CTF1 demonstrated high resistance to kanamycin, streptomycin, penicillin, moderate resistance to erythromycin, ampicillin, and chloramphenicol as well as weak resistance to tetracycline.

Table 2. Bacterial identification and PGP-attributes of bacterial strain CTF1.

Closest relative sequence	[1]Percentage of similarity	[2]IAA production (mg/L)	[2]Phosphate solubilization (mg PO_4^{3-}/L)	[3]ZnO-solubilization ratio	[3]Siderophore production ratio
Arthrobacter sp.	99.47%	12.22 ± 0.25	15.58 ± 1.02	2.50	3.60

Note: [1]Similarity with the strains of NCBI database. [2]Data are presented as Means ± SE (n = 3). [3]Ratio of halo zone + colony to colony diameter. IAA – indole-3-acetic acid.

The percentage of pea seed germination varied from 93.8 to 100% and averaged 95.7 and 98.9% for non-inoculated and inoculated plants, respectively. However, no significant differences were found between the treatments. The length of aboveground part of 7-day-old seedlings inoculated with bacteria Arthrobacter sp. strain CTF1 was 32% lower compared to non-inoculated ones, while no significant differences among all treatments were found in the length of 14-day-old shoots (averaged 10.4 cm, Fig. 1A). At the same time folia spraying with iodine did not have a considerable effect on the length of the 14-day-old seedlings (Fig. 1A).

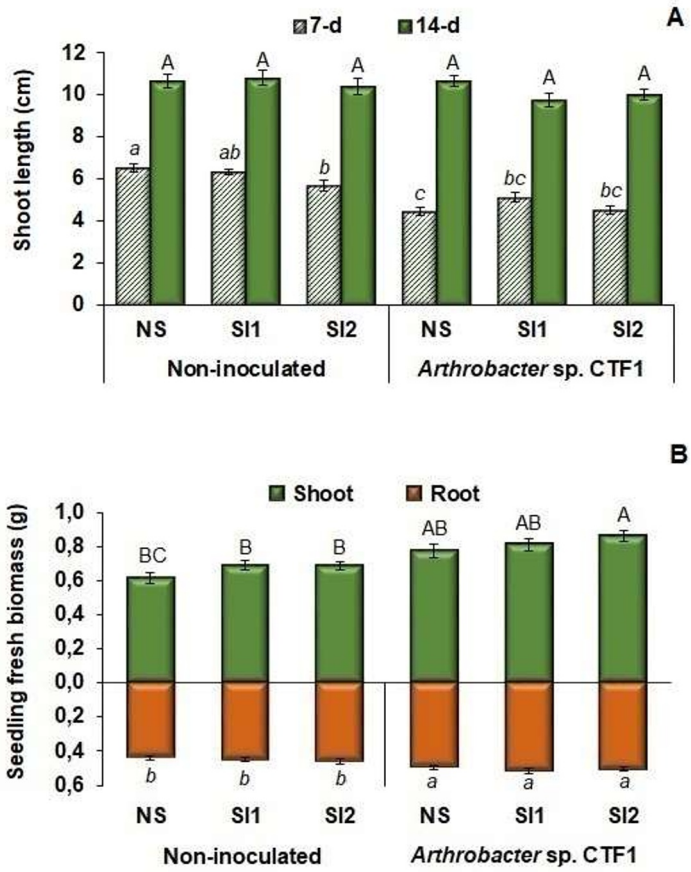

Fig. 1. Non-inoculated and inoculated by *Arthrobacter* sp. strain CTF1 pea seedling length (A) and its fresh biomass (B) after folia spraying with 0.01% KI (SI1) or KIO_3 (SI2). 7-d – 7-day-old seedlings; 14-day-old seedlings; NS – no spraying. Data are presented as Means ± SE (n = 30). Different alphabetical letters indicate significant difference between treatments according Tukey's test ($p < 0.05$).

The inoculation of pea with *Arthrobacter* sp. strain CTF1 increased fresh biomass of shoot and root by 21 and 12%, respectively, on average compared to control (Fig. 1B). Simultaneously, a direct dependence of the vigor index on the dry biomass of 14-day-old seedlings was discovered (r = 0.99, $p < 0.0001$), while both parameters increased considerably in inoculated plants (Fig. 2). The revealed effect is consistent with the data of other authors [22, 23]. For example, previously it was also reported that inoculation of rice with *Arthrobacter* sp. significantly increased its biomass [23]. Spraying *P. sativum* seedlings with either potassium iodide (SI1) or potassium iodate (SI2) did not significantly affect the

fresh biomass of both inoculated and non-inoculated plants (Fig. 1B). However, the vigor index in CTF1 inoculated plants after folia spraying with iodine was higher by 15% than without spraying, and by 32% in comparison with non-inoculated pea seedlings (Fig. 2).

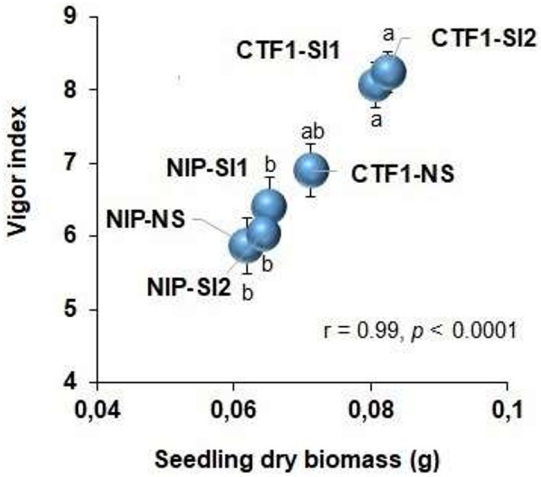

Fig. 2. Dependence of the vigor index on the dry weight of 14-day-old pea seedlings after folia spraying with 0.01% KI (SI1) or KIO_3 (SI2). NIP – non-inoculated plants; NS – no spraying; CTF1 – *Arthrobacter* sp. strain CTF1. Data are presented as Means ± SE (n = 30). Different alphabetical letters indicate significant difference between treatments according Tukey's test ($p < 0.05$).

Inoculation of pea with *Arthrobacter* sp. strain CTF1 increased the content of chlorophylls (Chl *a* and Chl *b*) by an average of 23% and Car by 37% in the leaves of 14-day-old microgreens (Fig. 3 A). At the same time, treatment of pea-inoculated seedlings with iodine (regardless of its form) had a significant effect only on the content of Car (by 18% higher compared to the control without spraying). The ratio of Chl *a* to Chl *b* in all treatments remained at a constant level (1.7 on average). Simultaneously, the ratio of Chl (*a*+*b*) to Car (6.0 on average) decreased significantly (by 17%) in inoculated plants at iodine application due to a considerable increase in Car content (Fig. 3B). It is known that Car are not only auxiliary photosynthetic pigments and important photoprotectors but also, they play an important role in reducing the negative effect of reactive oxygen species not only in plants, but also in humans [25]. An increase in antioxidant activity in plants in the presence of the *Arthrobacter* sp. strain was previously reported by Platamone et al. [22].

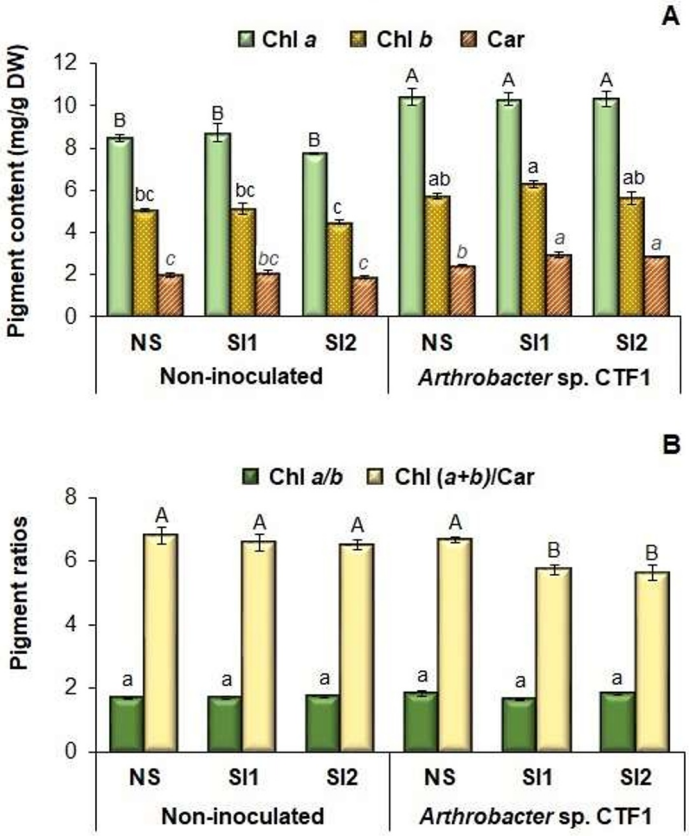

Fig. 3. Photosynthetic pigment content (A) and pigment ratios (B) in the leaves of 14-day-old non-inoculated and inoculated by *Arthrobacter* sp. strain CTF1 pea seedlings after folia spraying with 0.01% KI (SI1) or KIO_3 (SI2). NS – no spraying; Chl – chlorophyll; Car – carotenoids. Data are presented as Mean ± SE (n = 4). Different alphabetical letters indicate significant difference between treatments according Tukey's test ($p < 0.05$).

In control plants, the iodine content was low and averaged 3.5 ± 0.5 mg/kg DW. Folia spraying ~~of~~ *P. sativum* with iodine increased its content in the seedlings by an average of 26 times. However, neither the form of iodine (KI or KIO_3) nor the bacterial inoculation of *Arthrobacter* sp. strain CTF1 created any significant effect on its concentration in pea microgreens (Fig. 4). Jerše et al. [13] also reported that the iodine content in all organs of peas after iodine (KI or KIO_3) seed treatment increased significantly regardless of the form of iodine. However, a study of lettuce in a hydroponic growing system showed that the iodine content in leaves using potassium iodide was significantly (5 times) higher compared to potassium iodate [11].

A two-way ANOVA test showed that namely bacterial inoculation with CTF1 had the greatest effect on the most morphophysiological parameters, while the folia spraying with iodine (KI or KIO_3) had the significant impact only on 7-day-old seedling length, vigor index, Chl *b* and Car content (Table 3). At the same time the conjugate influence of both factors (iodine spraying and bacterial inoculation) had a significant effect only on the 7-day-old seedling length and Car content (Table 3).

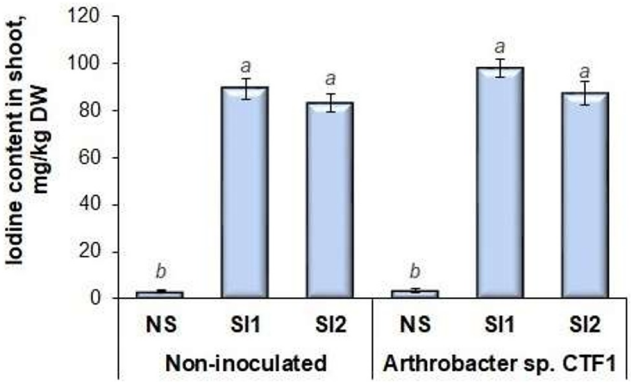

Fig. 4. Iodine content in the underground part of 14-day-old non-inoculated and inoculated by *Arthrobacter* sp. strain CTF1 pea seedlings after folia spraying with 0.01% KI (SI1) and KIO_3 (SI2). NS – no spraying. Data are presented as Mean ± SE (n = 4). Different alphabetical letters indicate significant difference between treatments according Tukey's test ($p < 0.05$).

Table 3. Results of two-way analysis of variance (ANOVA) of pea seedling parameters.

Parameter	Factor					
	Bacterial inoculation		Iodine spraying		Iodine spraying × Bacterial inoculation	
	F	*p*	F	*p*	F	*p*
7-day-old seedling length	80.39	0.000*	3.33	0.008*	3.33	0.039*
14-day-old seedling length	2.97	0.087	1.30	0.350	1.30	0.280
Seedling fresh biomass	32.26	0.000*	0.34	0.064	0.34	0.716
Seedling dry biomass	28.21	0.000*	0.90	0.088	0.90	0.418
Vigor index	38.18	0.000*	1.67	0.028*	1.67	0.210
Chl *a* content	66.79	0.000*	1.42	0.265	1.42	0.268
Chl *b* content	41.34	0.000*	1.09	0.011*	1.09	0.358
Car content	116.43	0.000*	5.38	0.008*	5.38	0.015*

Note: Chl – chlorophyll, Car – carotenoids. *Significant results.

4 Conclusion

The results obtained elucidate that the inoculation of *Pisum sativum* seeds with PGPR *Arthrobacter* sp. strain CTF1 had a positive effect on the fresh and dry biomass of two-week-old seedlings and their vigor index. At the same time, a significant increase in photosynthetic pigments was also observed in the leaves of pea microgreens, especially chlorophyll *a* (by almost 25%) and carotenoids (by almost 40%). It was noted that foliar spraying with iodine, regardless of its form (KI or KIO_3), led to an almost 26-fold increase in its amount in pea microgreens. However, spraying with iodine had a significant positive effect only on the content of carotenoids. The further investigations are still needed to better understand the mechanisms of combined biofortification using PGPR and iodine, as well as to identify its optimal concentrations that provide the greatest enrichment of microgreens with essential elements and biologically active substances such as carotenoids, vitamins, flavonoids and others. This will allow to solve the food problem associated with the provision of complete and balanced food products.

The work was supported and funded by Russian Science Foundation, Project No. 23 -26-00292, https://rscf.ru/project/23-26-00292.

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