Maleva, M., Borisova, G., Tripti, Tugbaeva, A., Ahamuefule, C., Salata A., Kumar, A. (2024). Biofortification of pea microgreens through zinc-solubilizing bacteria inoculation with foliar iodine application. Agriculture and Forestry, 70 (2): 123-134. https://doi.org/10.17707/AgricultForest.70.2.09

DOI: 10.17707/AgricultForest.70.2.09

Maria MALEVA*[1], Galina BORISOVA[1], TRIPTI[1],
Anastasia TUGBAEVA[1], Chrisantus AHAMUEFULE[1],
Amjed SALATA[1], Adarsh KUMAR[2]

BIOFORTIFICATION OF PEA MICROGREENS THROUGH ZINC-SOLUBILIZING BACTERIA INOCULATION WITH FOLIAR IODINE APPLICATION

SUMMARY

Biofortification of plant food products including microgreens is one of the most promising approaches to combat with the problem of balanced nutrition for the human population. This study introduces the microbiological biofortification using plant growth-promoting (PGP) bacteria, specifically three strains of zinc-solubilizing *Pseudomonas* sp. (STF10, STF14, STF16) isolated from the rhizosphere of *Tussilago farfara* L. The research explores their impact on growth parameters and photosynthetic pigments in pea (*Pisum sativum* L.) microgreens following iodine spraying, providing groundbreaking insights. The experimental results indicate that inoculation of pea seeds with these rhizobacteria significantly increased the fresh and dry biomass of 14-day-old microgreens (by 22 and 15%, respectively, on average) compared to non-inoculated plants. At the same time the content of chlorophylls and carotenoids in seedlings increased on average by 18 and 36%, respectively. Spraying with one form of iodine (0.01% KI or KIO_3) slightly increased fresh and dry biomass (by 8% on average) and did not alter the content of photosynthetic pigments. Wherein iodine content in pea microgreens increased by 4.4 and 3.8 times, after KI and KIO_3 spraying, respectively. By conducting experiments in a hydroponic nutrient solution, this study provides a robust platform for evaluating the biofortification potential of the three *Pseudomonas* strains along with foliar iodine feeding, marking a significant stride towards enhancing the nutritional profile of pea microgreens.

Keywords: *Pisum sativum* L., *Pseudomonas* sp., biofortification, micronutrient deficiency, plant morphophysiological traits, iodine accumulation

[1]Maria Maleva, Galina Borisova, Tripti, Chrisantus Ahamuefule, Anastasia Tugbaeva, Amjed Salata (corresponding author: maria.maleva@mail.ru), Ural Federal University named after the first President of Russia B.N. Yeltsin, Ekaterinburg 620002, RUSSIA

[2]Adarsh Kumar, Gandhi Institute of Technology and Management, GITAM School of Science, Department of Life Sciences, Visakhapatnam 530 045, Andhra Pradesh, INDIA

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
Received:15/02/2024 *Accepted:11/06/2024*

INTRODUCTION

The problem of micronutrient deficiency in the dietary habits of human populations holds significant importance for both developing and developed countries (Medrano-Macías *et al.*, 2016; Gharibzahedi and Jafari, 2017; Van Der Straeten *et al.*, 2020; Golubkina *et al.*, 2021). One of the most promising approaches to reduce global malnutrition is biofortification, or the enrichment of plant raw materials and crop products, including microgreens (White and Broadley, 2009; Eliseeva *et al.*, 2022; Sarwar *et al.*, 2022; Ahmad *et al.*, 2023). Currently, zinc (Zn), iron (Fe), and iodine (I) stand out as the three most crucial micronutrients from a global public health perspective, with their deficiency posing a serious threat to public health worldwide (Medrano-Macias *et al.*, 2016; Gharibzahedi and Jafari, 2017; Van Der Straeten *et al.*, 2020; Şahin, 2020; Singh and Prasanna, 2020; Ahmad *et al.*, 2023).

Biofortification, which was carried out by feeding crops with various micronutrients, became prevalent (Voogt *et al.*, 2010; Smoleń *et al.*, 2016; Jerse *et al.*, 2017). The experiments on the enrichment of various crops with micronutrients by adding them to the soil or folia spraying were carried out by many authors. Some of scientists studied the effect of biofortification using one element (Voogt *et al.*, 2010; Weng *et al.*, 2013), whereas others used two elements (Smoleń *et al*., 2016; Jerše *et al.*, 2017; Golubkina *et al.*, 2021). Data on the use of the three elements is fragmentary. For example, Şahin (2020) determined the effect of combined I-Fe-Zn treatments of tomato and reported that biofortification is an important way to eliminate the deficiency of these three elements in plants.

In recent years, microbial biofortification has garnered considerable interest, focusing on using various microorganisms to enrich crops with essential elements (Sunithakumari *et al.*, 2016; Ahmad *et al.*, 2023). Microbe-assisted biofortification attracted much attention recently due to its sustainable and eco-friendly nature for improving nutrient content (Sun *et al.*, 2021). Plant growth-promoting rhizobacteria (PGPR) emerge as a promising technique particularly biofortification, possessing capabilities such as nitrogen fixation, solubilization of phosphorus, potassium, iron, zinc, etc. that are inaccessible to plants, and production of siderophores and phytohormones (Saravanan *et al*., 2003; Kumar *et al*., 2021; Aloo *et al.*, 2022; Saleem *et al*., 2022; Yadav *et al.*, 2022).

Recent studies have reported the use of microorganisms for micronutrient biofortification of crops, but most authors have studied fortification of only one element. Hussain *et al.* (2015) studied prospects of zinc-solubilizing bacteria for enhancing growth of maize. Yadav *et al.* (2022) fortified wheat with zinc using Zn-solubilizing bacteria. Sarwar *et al.* (2021) reported the feasibility of iron biofortification using siderophore producing rhizobacteria strains to improve growth, yield and quality of groundnut. Daliran *et al.* (2022) found *Thiobacillus* sp. bacteria that could enhance iron biofortification of soybeans in limestone soil with elevated levels of ferrous sulfate. In recent years, biofortification with two or more microelements using PGP-bacteria has gained particular interest, however, positive results of "combined" biofortification with all studied micronutrients were

reported only in a few studies. For instance, Ahmad *et al.* (2023) isolated the strains of Zn-solubilizing and siderophore producing bacteria from the genera *Bacillus* and *Paenibacillus* and studied their biofortification potential for maize.

The challenge of enriching plant materials and food products, including microgreens, with essential micronutrients is relevant globally. However, despite its potential, microbial biofortification has not seen widespread application, and the exploration of combined biofortification possibilities remains limited, motivating the objectives of our research.

Thus, the study aims to assess the zinc-solubilizing potential and PGP attributes of three rhizobacteria strains (genus *Pseudomonas*), as well as their effect on growth parameters and the content of photosynthetic pigments in pea microgreens after spraying with iodine.

MATERIAL AND METHODS

To isolate the rhizosphere bacteria, one of the most dominant plant species *Tussilago farfara* L. (Asteraceae family) growing on the disturbed territory close to Safyanovsky copper mine (Rezh, Sverdlovsk region, Russia) was selected. The rhizosphere soil adhered to root strands of a random selection of three flowering plants (at the end of May 2023) was collected by shaking them gently in a zip-lock bags and transferred to the laboratory. About 10 g of rhizosphere soil was mixed with 90 mL of phosphate buffer (pH 6.5) and shaken at 180 rpm for 2 h at 28 °C. A series of dilutions of each sample were made and 100 μL was added to a Petri plate with Luria-Bertani (LB) agar supplemented with 100 mg/L Zn (sulfate form). A total of 16 cultures were isolated from soil and subjected on MSM agar (Bhakat *et al.*, 2021) supplemented with 0.1% of $ZnCO_3$, $Zn_3(PO_4)_2$ and ZnO for 2 days at 28 °C. 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 (Eshaghi *et al*., 2019).

Siderophore production was assessed as described earlier (Kumar *et al.*, 2021) after interaction of rhizobacteria culture (10^8 cfu/mL) with Fe-CAS (chromazurol S) indicator solution and was identified by orange to yellow halo zones around bacterial colonies. Siderophore production ratio was calculated as diameter of solubilized halo zone to the diameter of the colony.

The iodine test was performed to understand the strain resistance level. The iodine rich LB agar plates were prepared, and all isolates were subjected to an increasing concentration of two form 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.

Following the screening of bacterial isolates for their zinc solubilization, siderophore production, and iodine resistance, three strains were singled out for in-depth analysis through morphological, physiological, and molecular genetic identification. The selected strains underwent Gram staining to determine their Gram-positive or Gram-negative nature, and their morphological properties such

as shape, size, texture, etc. were assessed. For genus identification, molecular genetic analysis was conducted using 16S rRNA (rDNA) genome sequencing (Voropaeva *et al.*, 2022). Genomic DNA from liquid cultures of rhizobacteria was isolated utilizing spin columns, following the guidelines provided by the manufacturer (DiaGene kit 3318.0250, Dia-M LLC, Russia). The total DNA (10 ng) was used as a template for the amplification of 16S rRNA genes using 16S Barcoding Kit (SQK-16S 024, Oxford Nanopore Technologies, UK) and LongAmp Hot Start Taq 2 × Master Mix (New England Biolabs, USA). The PCR product was purified using AMPure XP (Beckman Coulter, USA) and used to prepare a sequencing library (SQK-16S024, Oxford Nanopore Technologies, UK). Sequencing was performed on a GridION^{TM} sequencer in an R9.4 flow cell (Oxford Nanopore Technologies, UK). The computer programs of Oxford Nanopore Technologies were used for data analysis. Primary data processing was carried out in MINKNOW software ver. 21.05.8. The raw FAST5 files were base called using Guppy version 5.0.11 to generate FASTQ files. The EPI2ME Fastq 16S ver. 3.3.0 were used for systematic classification of 16S rRNA gene.

The selected isolates were tested for phosphate solubilization, indol-3-acetic acid (IAA), and ammonia production as described previously (Kumar *et al.*, 2021). 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 at 420 nm using UV-Vis spectrophotometer (Teccan, Thermo Scientific, USA). The ability to solubilize phosphates was assessed by preparing a calibration curve based on the standard solution of potassium dihydrogen orthophosphate and expressed in mg PO_4^{3-}/L. The IAA production was checked by appearance of pink color after adding Salkowski's reagent to freshly prepared bacteria cultures (10^8 cfu/mL) and measured at 530 nm. Commercially available IAA (Sigma-Aldrich) was used to prepare a calibration curve. 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.

Antibiotic resistance of the selected strains was checked by disc diffusion method (Rajkumar *et al.*, 2013). 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).

The influence of selective strains on the germination of pea seeds (*Pisum sativum* L., Madras var.) was assessed in Petri plate experiment. The mature seeds similar in size and shape were surface sterilized (by 70% ethanol for 30 s, then 2 min with 4% sodium hypochlorite), and finally multiple washing using sterile Millipore water (Millipore, USA). The seeds were soaked overnight and were inoculated for 2 hours with the selective bacterial cultures (10^8 cfu/mL) pre-grown on LB medium, while in the control (CS) it was only sterile LB medium. A total of 36 seeds were placed to each Petri plate (10 plates in each treatment), equipped with moistened sterile filter paper at the bottom, and seed germination was checked

daily until full sprouting. After that, the seedlings were transferred to the plastic sprouters ("The home AeroGARDEN", SmartGidroCompany, LLC, Russia) with 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 s provided by phytolamps (ULI-P10-18W/SPFR IP40); day/night regime of 14/10 at 23 ± 3 °C. Upon the emergence of the first leaves, the seedlings were sprayed with 0.01% KI (SI1) or KIO_3 (SI2) or just sterile water without iodine (NS). Every treatment included 3 independent boxes with 40 pea seedlings in each.

Germination characteristics were calculated according to Kumar *et al.* (2012). Germination percentage (%) was calculated as total number of seeds germinated at the end of counting days/total number of seeds × 100%; seedling vigor index was calculated as germination percentage (%) × mean seedling dry biomass (g). Growth parameters such as length, fresh biomass (FW) and dry biomass (DW), and photosynthetic pigment content were measured in 14-day-old pea seedlings. 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 per one gram of DW according to Lichtenthaler (1987). The iodine content in dry pea seedling biomass (shoot) was determined according to GOST 28458-90 (2006) and expressed in mg/kg DW.

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 alphabetical letters indicate significant difference between treatments at $p < 0.05$.

RESULTS AND DISCUSSION

Three bacterial cultures, namely STF10, STF14, and STF16, isolated from the rhizosphere of *T. farfara*, were assessed for their capacity to solubilize various insoluble forms of zinc (Table 1). The best Zn solubilization was noted for the strain STF14, while STF10 and STF16 better solubilized zinc oxide or zinc carbonate. Furthermore, an examination of siderophore production revealed that, after a 3-day incubation period, all isolates demonstrated approximately the same level of siderophore production (Table 1).

The iodine test with KI and KIO_3 revealed no adverse effects on the growth of the studied strains, qualifying them for potential use in plant biofortification (Table 1). The Gram staining test showed that all three isolates were negative. Morphologically, STF14 and STF16 exhibited similarities in colony characteristics, while STF10 differed in traits such as shape, size, and pigmentation (Table 2). In addition, all strains showed fast growing properties which are very useful for conducting the experiment.

Table 1. Zinc solubilization, siderophore production and iodine resistance of selected bacteria strains isolated from rhizosphere of *T. farfara*

Strain	Zn solubilization ratio*			Siderophore production ratio*	Iodine resistance	
	ZnO	$ZnCO_3$	$Zn_3(PO_4)_2$		KI	KIO_3
STF10	3.0	2.8	2.0	1.4	High	High
STF14	3.8	3.0	2.8	1.4	High	High
STF16	1.2	2.5	2.2	1.5	High	High

*Ratio of halo zone + colony to colony diameter

Table 2. Morphological properties of selected bacteria strains isolated from rhizosphere of *T. farfara*

Strain	Morphological characteristics of colonies (on solid LB medium)					
	Shape	Margin	Elevation	Size	Texture	Pigmentation
STF10	Irregular	Entire	Raised	Large	Smooth	Pale brown
STF14	Circular	Entire	Raised	Moderate	Smooth	Brown
STF16	Circular	Entire	Raised	Moderate	Smooth	Brown

The isolates were identified with 16S rRNA sequencing as *Pseudomonas* sp. with 93.6–94.7% similarity (Table 3). It was previously noted that most zinc solubilizing bacteria belongs to genera *Pseudomonas*, *Bacillus*, *Enterobacter*, *Xanthomonas*, and *Stenotrophomonas* (Saravanan *et al.*, 2003; Hussain *et al.*, 2015; Sunithakumari *et al.*, 2016).

Further, the studied strains were tested for additional PGP attributes (Table 3). All strains were able to solubilize insoluble phosphates and produce IAA. The maximum values of these PGP traits were found in strain STF14. The ammonia production was also noted for all studied *Pseudomonas* sp. strains (Table 3). Moreover, these strains demonstrated high or moderate resistance to erythromycin, tetracycline, ampicillin, chloramphenicol as well as penicillin. However, they turned out to be susceptible (highly or moderate) to kanamycin and streptomycin.

Table 3. Bacterial identification and plant growth promoting attributes of selected bacteria strains isolated from rhizosphere of *T. farfara*

Strain	Closest relative sequence	[1]Percentage of similarity/ Number of reads	[2]Phosphate solubilization, mg PO_4^{3-}/L	[2]IAA production, mg/L	NH_3 production
STF10	*Pseudomonas* sp.	94.7/35098	10.4 ± 0.5b	3.9 ± 0.8c	+
STF14	*Pseudomonas* sp.	94.4/83713	34.8 ± 0.9a	31.8 ± 0.8a	+
STF16	*Pseudomonas* sp.	93.6/26200	5.5 ± 0.5c	12.4 ± 0.6b	+

[1]Similarity with the strains of NCBI database. [2]Data are presented as Means ± SE (n = 3). IAA – indole-3-acetic acid

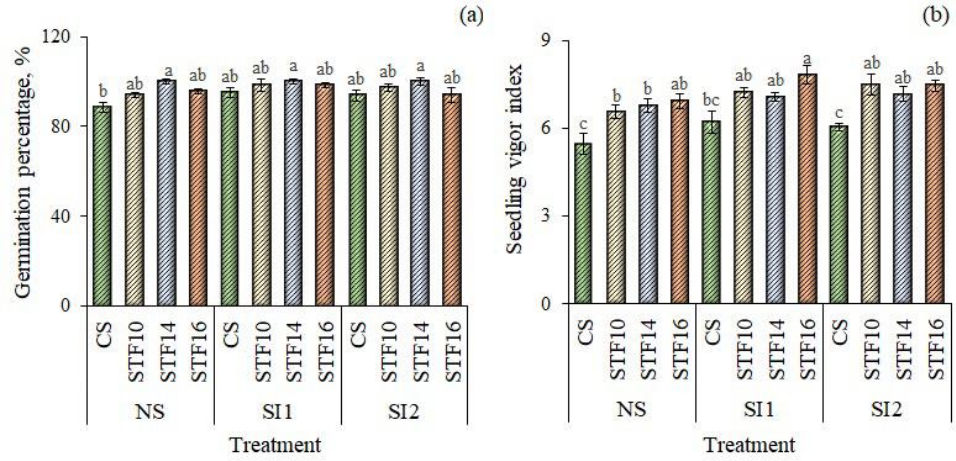

Fig. 1. The germination percentage (a) and vigor index (b) of *P. sativum* seedlings after folia spraying with 0.01% KI (SI1) or KIO_3 (SI2). Data are presented as Means ± SE (n = 10). CS – control seedlings; NS – no spraying

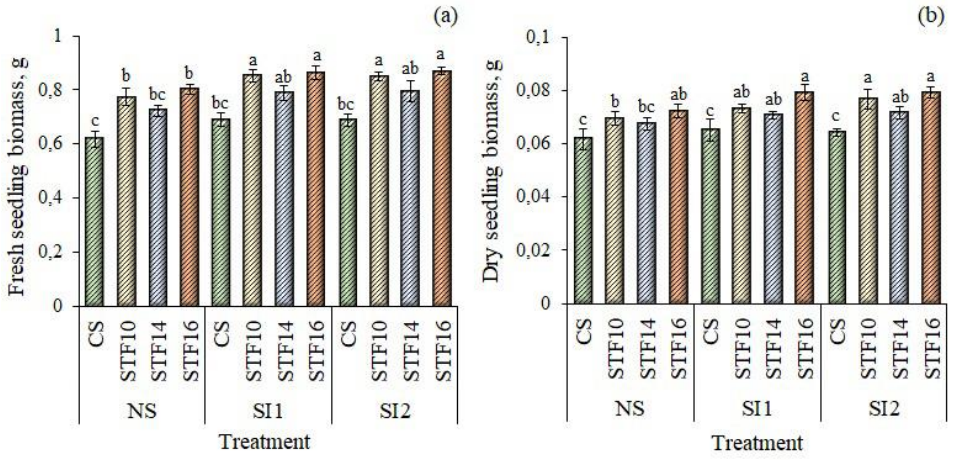

Fig. 2. The fresh (a) and dry (b) biomass of 14-day-old *P. sativum* seedlings after folia spraying with 0.01% KI (SI1) or KIO_3 (SI2). Data are presented as Means ± SE (n = 20). CS – control seedlings; NS – no spraying

The percentage of seed germination is one of the most important characteristics of the crops. Only those seeds that germinate rapidly and vigorously under favorable conditions in the laboratory are likely to produce vigorous seedlings in the field (Kumar *et al.*, 2012). The percentage of seed germination in all treatments varied from 88.3% (in control without iodine spraying) to 100% (in pea seeds inoculated STF14) (Fig. 1a). Moreover, in all inoculated plants the seedlings vigor index significantly increased (by 20% on average) in comparison with non-inoculated control (Fig. 1b). Seed vigor is the sum total of those

properties of the seed that determine the level of activity and performance of the seeds during germination and seedling emergence (Kumar *et al*., 2012).

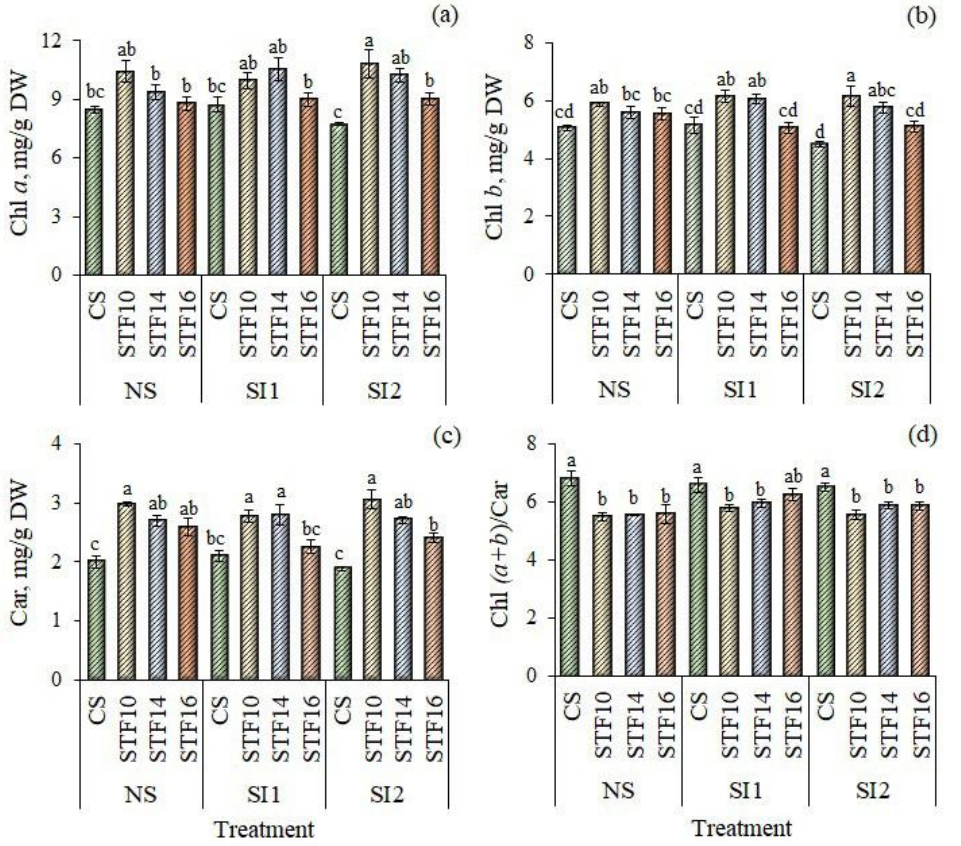

Fig. 3. The content of chlorophyll *a* (a), chlorophyll *b* (b), carotenoids (c) and ratio of total chlorophyll to carotenoids in leaves of 14-day-old *P. sativum* seedlings after folia spraying with 0.01% KI (SI1) or KIO_3 (SI2). Data are presented as Means ± SE (n = 4). CS – control seedlings; NS – no spraying

No significant differences among all treatments were found in the length of 14-day-old seedlings (averaged 10.7 cm), while fresh and dry biomass of inoculated seedlings increased on average by 22 and 15%, respectively, compared to control (Fig. 2 a,b). The revealed effect is consistent with the data of other authors (Hussain *et al.*, 2015; Ahmad *et al.*, 2023), reported that the zinc solubilizing bacteria significantly improved the growth of maize seedlings.

Spraying *P. sativum* seedlings with either potassium iodide (SI1) or potassium iodate (SI2) did not significantly affect the biomass of non-inoculated plants (Fig. 2 a,b). However, spraying with iodine slightly increased the biomass of inoculated seedlings (by 8% on average).

Inoculation of pea with bacteria increased the content of Chl *a* and Chl *b* in seedlings by 18% on average (Fig. 3 a,b), and Car by 36% (Fig. 3 c). Moreover, in

terms of their effect on photosynthetic pigments, the studied PGPR strains followed the descending sequence: STF10 > STF14 >STF16. The strain STF10 had the best positive effect on the content of chlorophylls (25% on average) and carotenoids (48% on average). The positive effect of zinc solubilizing bacteria from the other genus (*Bacillus*) on the chlorophyll content in maize seedlings was also noted in other study (Hussain *et al.*, 2015).

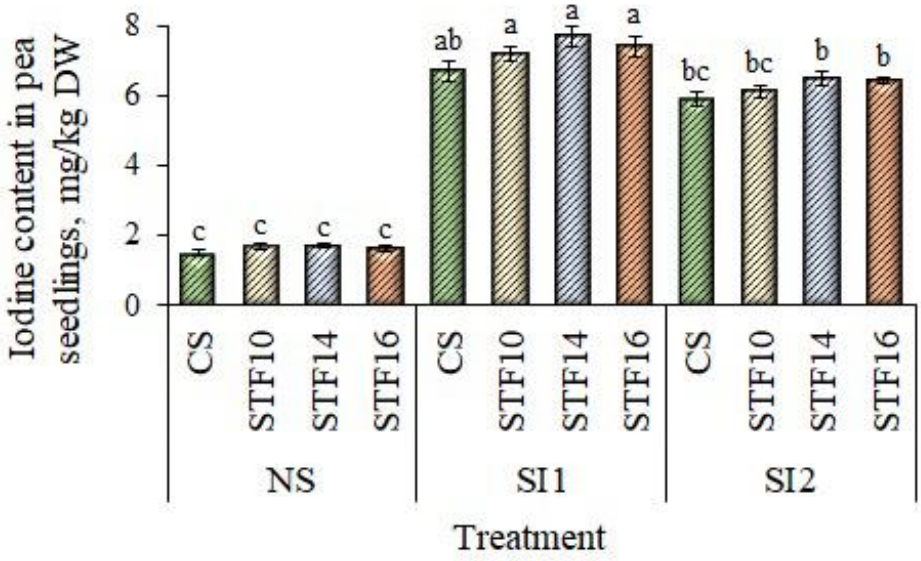

Fig. 4. Iodine accumulation in the 14-day-old *P. sativum* seedlings (shoot) after folia spraying with 0.01% KI (SI1) or KIO_3 (SI2). Data are presented as Means ± SE (n = 3). CS – control seedlings; NS – no spraying

At the same time, the ratio of Chl *a* to Chl *b* in all treatments remained at a constant level (about 3 on average), and the ratio of Chl (*a*+*b*) to Car in inoculated plants decreased significantly due to a considerable increase in carotenoids (Fig. 3 d). It is known that Car are auxiliary photosynthetic pigments and important photoprotectors that defend chlorophyll molecules from oxidation. In addition, they play an important role in reducing the negative effect of reactive oxygen species not only in plants, but also in humans. Thus, Car are bioactive substances in human diet with powerful antioxidant activity (Dymova *et al.*, 2014).

Spraying the leaves of *P. sativum* seedlings with iodine did not affect the pigment content (Fig. 3 a,b,c). This observation aligns with earlier findings by Jerše *et al.* (2017), who also reported no positive effects of iodine on the biomass and photosynthetic pigment content of pea seedlings. Notably, our experiments revealed that the pivotal factor influencing these parameters was the inoculation of pea seeds with Zn-solubilizing rhizobacteria, rather than spraying with iodine, which was confirmed by a two-way ANOVA ($F_{FW} = 30.1$; $F_{DW} = 12.3$; $p < 0.001$ and $F_{Chl} = 5.9$; $F_{Car} = 12.7$; $p < 0.001$).

The iodine content in pea biomass treated with potassium iodide and iodate increased by 4.4 and 3.8 times respectively, compared to the NS-treatment (Fig. 4). At the same time, the iodine accumulation in microgreens didn't depend on type of rhizobacteria strain, however, differ in dependence on the form of iodine: SI1 enhanced iodine content by 16% higher compared to SI2. Previously Voogt *et al.* (2010) also reported that the iodine content in lettuce leaves in a hydroponic growing system using potassium iodide was significantly higher compared to potassium iodate.

CONCLUSIONS

The zinc-solubilization potential and other PGP attributes of three rhizobacteria strains *Pseudomonas* sp. (STF10, STF14, STF16) had been reported for the first time in this study. The experimental results indicate that inoculation of pea seeds with these PGP-rhizobacteria significantly enhanced the fresh and dry biomass of *Pisum sativum* microgreens as well as the content of chlorophylls and especially carotenoids compared to non-inoculated control seedlings. Among the strains, inoculation with the STF10 strain resulted in the most substantial increase in the content of photosynthetic pigments in pea seedlings. The folia spraying of inoculated *P. sativum* seedlings with iodine slightly increased fresh and dry biomass and did not change the photosynthetic pigment content. The seedlings spraying with KI or KIO_3 increased its content in pea microgreens by 4.4 and 3.8 times, respectively, as compared to the non-spraying treatment. Thus, experiments in hydroponic nutrient solution made it possible to evaluate the biofortification potential of three isolated *Pseudomonas* strains along with foliar iodine feeding. However, it is essential to conduct further investigations, particularly in pot-scale experiments, to validate the positive effects observed with the studied strains and iodine spraying.

ACKNOWLEDGEMENTS

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

REFERENCES

Ahmad, M., Hussain, A., Dar, A., Luqman, M., Ditta, A., Iqbal, Z., Ahmad, H. T., Nazli, F., Soufan, W., Almutairi, K. & Sabagh, A. E. (2023): Combating iron and zinc malnutrition through mineral biofortification in maize through plant growth promoting *Bacillus* and *Paenibacillus* species. *Front Plant Sci*, 13: 1094551.

Aloo, B. N., Tripathi, V., Makumba, B. A. & Mbega, E. R. (2022): Plant-growth promoting rhizobacterial biofertilizers for crop production: The past, present, and future. *Front Plant Sci*, 13: 1002448.

Bhakat, K., Chakraborty, A. & Islam, E. (2021): Characterization of zinc solubilization potential of arsenic tolerant *Burkholderia* spp. isolated from rice rhizospheric soil. *World J Microbiol Biotechnol*, 37: 39.

Daliran, T., Halajnia, A. & Lakzian, A. (2022): *Thiobacillus* Bacteria-Enhanced Iron Biofortification of Soybean in a Calcareous Soil Enriched with Ferrous Sulfate, Mill Scale, and Pyrite. *J Soil Sci Plant Nutr*, 1–14.

Dymova, O., Lashmanova, E. & Golovko, T. (2014): Plant pigments and human health. *In* T. K. Golovko, W. I. Gruszecki, M.N.V. Prasad & K. Strzalka, eds. *Photosynthetic pigments: chemical structure, biological function and ecology*, pp. 423–438. Syktyvkar. Komi Scientific Centre of the Ural Branch of the Russian Academy of Sciences. 448 pp.

Eliseeva, L. G., Belkin, Yu. D., Simina, D. V., Osman, A., Molodkina, P. G. & Santuryan, T. A. (2022): New trajectories in the development of fortified foods for a healthy diet. *Int Res J*, 4(118): 50–55 (In Russian).

Eshaghi, E., Nosrati, R., Owlia, P., Malboobi, M. A., Ghaseminejad, P. & Ganjali, M.R. (2019) Zinc solubilization characteristics of efficient siderophore-producing soil bacteria. *Iran J Microbiol*, 11(5): 419–430.

Gharibzahedi S.M.T. & Jafari S. M. (2017): The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. *Trends in Food Sci Technol,* 62: 119e132.

Golubkina, N., Moldovan, A., Kekina, H., Kharchenko, V., Sekara, A., Vasileva, V., Skrypnik, L., Tallarita, A. & Caruso, G. (2021): Joint biofortification of plants with selenium and iodine. New field of discoveries. *Plants*, 10: 1352.

GOST 28458-90 (2006) *Vegetable feed, Method for determination of iodine*. Standardinform, Moscow (In Russian).

Hussain, A., Arshad, M., Zahir, Z. A. & Asghar, M. (2015): Prospects of zinc solubilizing bacteria for enhancing growth of maize. *Pak J Agric Sci*, 52(4): 915–922.

Jerše, A., Kacjan-Marsic, N., Šircelj, H., Germ, M., Kroflic, A. & Stibilj, V. (2017): Seed soaking in I and Se solutions increases concentrations of both elements and changes morphological and some physiological parameters of pea sprouts. *Plant Physiol Biochem*, 118: 285e294.

Kumar, B., Verma, S. K., Ram, G. & Singh, H. P. (2012): Temperature relations for seed germination potential and seedling vigor in Palmarosa (*Cymbopogon martinii*), *J Crop Improv*, 26: 791–801.

Kumar, A., Tripti, Voropaeva, O., Maleva, M., Panikovskaya, K., Borisova, G., Rajkumar, M. & Bruno, L.B. (2021): Bioaugmentation with copper tolerant endophyte *Pseudomonas lurida* strain EOO26 for improved plant growth and copper phytoremediation by *Helianthus annuus*. *Chemosphere*, 266: 128983.

Lichtenthaler, H. K. (1987) Chlorophylls and carotenoids: pigments of photosynthetic membranes. *Method Enzymol*, 148: 350–382.

Medrano-Macías, J., Leija-Martínez, P., González-Morales, S., Juárez-Maldonado, A. & Benavides-Mendoza, A. (2016): Use of iodine to biofortify and promote growth and stress tolerance in crops. *Front Plant Sci*, 7: 1146.

Rajkumar, M., Ma, Y. & Freitas, H. (2013): Improvement of Ni phytostabilization by inoculation of Ni resistant *Bacillus megaterium* SR28C. *J Environ Manag*, 128: 973–980.

Şahin, Ö. (2020): Combined iodine, iron and zinc biofortification of tomato fruit. *Soil Sci Plant Nutr*, 10(3): 2242–2251.

Saleem, M.H., Usman, K., Rizwan, M., Jabri, A.H. & Alsafran, M. (2022): Functions and strategies for enhancing zinc availability in plants for sustainable agriculture. *Front Plant Sci*, 13:1033092.

Saravanan, V. S., Subramoniam, S. R. & Raj, S. A. (2003): Assessing in vitro solubilization potential of different zinc solubilizing bacteria (ZSB) isolates. *Braz J Microbiol*, 34(1): 121–125.

Sarwar, S., Khaliq, A., Yousra, M. & Sultan, T. (2022): Iron biofortification potential of siderophore producing rhizobacterial strains for improving growth, yield and iron contents of groundnut. *J Plant Nutr*, 1–16.

Singh, D. & Prasanna, R. (2020): Potential of microbes in the biofortification of Zn and Fe in dietary food grains. A review. *Agron Sustain Dev*, 40: 15.

Smoleń, S., Skoczylas, Ł., Ledwożyw-Smoleń, I., Rakoczy, R., Kopeć, A., Piątkowska, E., Bieżanowska-Kopeć, R., Pysz, M., Koronowicz, A., Kapusta-Duch, J. & Pawłowski, T. (2016): Iodine and selenium biofortification of lettuce (*Lactuca sativa* L.) by soil fertilization with various compounds of these elements. *Acta Sci Pol Hortorum Cultus*, 15(5): 69–91.

Sun, Z., Yue, Z., Liu, H., Ma, K. & Li, C. (2021): Microbial-assisted wheat iron biofortification using endophytic *Bacillus altitudinis* WR10. *Front nutr*, 8: 476.

Sunithakumari, K., Padma Devi, S. N. & Vasandha, S. (2016): Zinc solubilizing bacterial isolates from the agricultural fields of Coimbatore. *Curr Sci*, 110(2): 196–205.

Van Der Straeten, D., Bhullar, N. K., De Steur, H., Gruissem, W., MacKenzie, D., Pfeiffer, W., Qaim, M., Slamet-Loedin, I., Strobbe, S., Tohme, J., Trijatmiko, K. R., Vanderschuren, H., Montagu, M. V., Zhang, C. & Bouis, H. (2020): Multiplying the efficiency and impact of biofortification through metabolic engineering. *Nat Commun*, 11(1): 1–10.

Voogt, W., Holwerda, H. T. & Khodabaks, R. (2010): Biofortification of lettuce (*Lactuca sativa* L.) with iodine: the effect of iodine form and concentration in the nutrient solution on growth, development and iodine uptake of lettuce grown in water culture. *J Sci Food Agric*, 90(5): 906–913.

Voropaeva, O. V., Borisova, G. G., Maleva, M. G., Podstavkina, A. V., Ermoshin, A. A., Tugbaeva, A. S. & Filimonova, E. I. (2022): Plant growth promoting activity and metal tolerance of bacteria isolated from rhizosphere of the orchid *Epipactis atrorubens* growing on serpentine substrates of the Middle Urals. *J Sib Feder Univ. Biology*, 15(3): 297–313 (In Russian).

Weng, H. X., Hong, C. L., Xia, T. H., Bao, L. T., Liu, H. P. & Li, D. W. (2013): Iodine biofortification of vegetable plants – An innovative method for iodine supplementation. *Chin Sci Bull*, 58(17): 2066–2072.

White, P. J. & Broadley, M. R. (2009): Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytol*, 182: 49–84.

Yadav, R. C., Sharma, S. K., Varma, A., Rajawat, M.V.S., Khan, M. S., Sharma, P. K., Malviya D., Singh U. B., Rai J. P. & Saxena, A. K. (2022): Modulation in biofertilization and biofortification of wheat crop by inoculation of zinc-solubilizing rhizobacteria. *Front Plant Sci*, 13: 777771.