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RESEARCH ARTICLE

Mitigating effect of PGPR on abiotic stress in basil (*Ocimum basilicum* L.)

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ABSTRACT

Having in mind food security and the fact that basil (*Ocimum basilicum* L.) is used as fresh seasoning, the development of innovative technologies for its cultivation is required. This study aimed to isolate and characterize plant growth-promoting rhizobacteria (PGPR) isolates from basil rhizospheric soil and monitor the effects of their application on basil growth under different water stress conditions. Isolation, determination of isolates biochemical and PGP properties, evaluation of isolates influences on seed germination and parameters of growth of basil plant, growing under well-watered conditions (70% water holding capacity, WHC), under water deficit stress (35% WHC), and flooded conditions (95% WHC), were done. A total of five representative bacterial isolates were selected: Two *Azotobacter* isolates (A13, A14), one *Pseudomonas* isolate (P57), and two *Bacillus* isolates (B79, B82). The results of this experiment revealed that rhizospheric bacteria of *O. basilicum* L. var. *minimum* have multiple biochemical and PGP properties. The most intensive reactions on tested abiotic stressors - drought (higher total phenolics, reduced glutathione, and malondialdehyde content) and flooding (higher superoxide dismutase activity) - were from basil plants inoculated with *Pseudomonas* sp. P57 isolate, yet along with *Azotobacter* sp. isolate A13. The highest number of germinated seeds was obtained with the A13 isolate (96.0%), while the highest response for vigour index was observed with *Azotobacter* isolates (7200.0% and 5628.0%). In well-watered conditions, basil mass inoculated with P57 was 34.2% higher than control. In drought-stressed conditions, plant mass inoculated with A13 isolate was 90% higher than control. In flooded-stressed conditions, the plant mass inoculated with *Azotobacter* and *Pseudomonas* isolates increased by more than 100%.

Key words: *Azotobacter* sp., basil, drought, flooding, oxidative stress, *Pseudomonas* sp.

INTRODUCTION

The basis for mitigating the effects of climate fluctuations on plant production is the implementation of adequate agrotechnical measures (Vogel et al., 2019). Depending on the cultivar and environmental conditions, these methods and technologies have their own specificities. Accordingly, good-quality agrotechnical measures ensure a high level of security and stability in primary plant production under climate variations. In recent years, a new range of alternative agricultural technologies have been developed to mitigate the effects of climate fluctuations in agriculture: Soil conservation measures, mulching, growing cover and mix crops, agroforestry, and others (Rodrigo-Comino et al., 2020). One of the great ways to overcome climate change issues is to introduce live plant growth-promoting rhizobacteria (PGPR) into the soil (Nivetha et al., 2021).

Recently, the use of plant growth-promoting rhizobacteria (PGPR) in the production of medicinal plants has gained increasing importance. In fact, in medicinal plants production, the use of PGPR has become crucial because agricultural chemicals, including various pesticides (herbicides, insecticides, and fungicides) and fertilisers, are no longer acceptable (Malik et al., 2011). For this reason, in the past few years, as interest in

medicinal herbs has grown, scientists have become increasingly interested in studying the diversity of microorganisms isolated from the rhizosphere of medicinal plants and the possibility of their use in the production of medicinal plants. Bafana and Lohiya (2013) investigated the endophytes and rhizospheric microorganisms from the root and rhizosphere of *Origanum vulgare*, while Kumar et al. (2012) investigated the rhizospheric microorganisms of medicinal plant *Ajuga bracteosa*. Also, the rhizosphere microbiome of many other medicinal plants was the object of research: *Ocimum* sp. (Verma and Saharan, 2020), *Angelica sinensis* (Lee et al., 2013), *Matricaria chamomilla* and *Calendula officinalis* (Köberl et al., 2013), and others. Results of many studies have shown that the use of PGPR in the production of medicinal herbs has a good impact on plant growth and enhanced harvest yield, increased herbal material quality, and raised plant resistance to abiotic stress (Jahanian et al., 2012; Mahdavikia et al., 2019; Cabanzo-Atilano et al., 2024).

Basil (*Ocimum basilicum* L.) is one of the most widely used medicinal plants belonging to the *Lamiaceae* family. It is naturally spread and cultivated throughout temperate climates. Basil has been cultivated for its essential oil, which benefits human health due to its antibacterial, antifungal, antiviral, anti-inflammatory, and relaxant properties. Their leaves are widely used as a seasoning or flavouring agent in the food, cosmetic, and perfume industries (Reyes-Pérez et al., 2021). The results of Copolovici et al. (2021) indicate that the water status (drought and/or flooding) directly impacts basil plants' physiological parameters and secondary metabolites. The results of very recent work by Hamidi et al. (2023) indicate that *Azospirillum* and *Azotobacter* can be considered a common strategy to maintain the growth of basil under water shortages.

Considering food security and the fact that basil is used and purchased as fresh seasoning (pots or other packages), innovative technologies for basil cultivation are required. Therefore, this study aimed to isolate and characterize PGPR isolates of *Bacillus*, *Pseudomonas*, and *Azotobacter* from basil rhizospheric soil and monitor the effects of their application on plant growth under well-watered, water-deficit, and flooded conditions.

MATERIALS AND METHODS

The basil rhizosphere microbiome was isolated from basil plants (*Ocimum basilicum* L. var. *minimum*) grown for seed and essential oil production at the Institute of Field and Vegetable Crops in Novi Sad (IFVCNS), National Institute of the Republic of Serbia, Department of Vegetable and Alternative Species. The seeds of 'Sitnolisni' basil were used in the experiment with abiotic stresses, as well.

Rhizosphere soil was collected during the summer in August 2019, when basil plants were in full flowering stage. The rhizosphere soil was sampled by picking three basil plants and separating the rhizospheric soil and roots from the rest of the plant in sterile plastic bags, after which the samples were transferred in a hand freezer to a regular freezer until further analyses.

Soil suspension (100 µL) was spread onto an appropriate selective medium and placed for incubation at 25 °C for 3 d. King-B medium was used for *Pseudomonas* sp., nutrient agar (NA) for *Bacillus* sp., and N-free medium for *Azotobacter* sp. isolation. Morphology characterization and identification of the isolates were examined using light microscopic observation (BA210, Motic, Hong Kong, China), while the taxonomy was based on reference books for bacteria identification (Holt, 1994).

Biochemical and PGP characterization of the isolates

Characterization of plant growth-promoting traits covered the determination of indol-3-acetic acid (IAA), siderophores, hydrogen cyanide (HCN) production and ACC-deaminase activity (Slimani et al., 2023). The ability of the mineralization of P organic compounds was assayed on Menkina medium (Liu et al., 2011), while the capacity of inorganic phosphate solubilization was examined on Pikovskaya's agar plates (Wahyudi et al., 2011). Enzyme production was tested according to the standard methods (Benson, 2002).

Evaluation of isolates for their PGP potential on basil plants

'Sitnolisni' basil seeds (IFVCNS) were used as plant material. The effect of isolates on seed germination and initial growth was examined under controlled conditions. Fifty seeds inoculated with the appropriate bacterial inoculate (seeds were submerged for 30 min in bacterial inocula, titre 10^9 CFU mL^{-1}) were placed on filter paper and put in a thermostat at 22 °C for germination. The seeds in the control were submerged for 30 min in an appropriate sterile nutrient broth medium. The number of germinated seeds was counted

after 3 and 7 d. The shoot and root lengths of germinated seeds were measured after 7 and 10 d. The vigour index (VI) was measured after 10 d and calculated as: VI (%) = (Mean root length + Mean shoot length) × Germination percentage.

Abiotic stress experiment

The experiment of drought and flooding effects on basil plants inoculated with the isolates was performed in 850 mL pots (10 cm bottom diameter, 13 cm upper diameter, and 11 cm height) filled with Klasmann substrate. The experiment had three treatments: 1) Well-watered, plants were watered to 70% water holding capacity [WHC]) (WW), 2) water deficit stress, 35% WHC (WDS), and 3) flooded conditions, 95% WHC (FC). Each treatment included basil plants in control (without PGPR), basil plants inoculated with *Azotobacter* sp. isolates (A13 and A14), *Bacillus* sp. isolates (B79 and B82), and *Pseudomonas* sp. (P57), respectively. The experiment was carried out in triplicate, completely randomized. Before sowing, 10 mL inocula of each selected isolates, titre 10^9 CFU mL^{-1}, were introduced into the substrate.

Plants were grown at room temperature (25 ± 2 °C) and under natural light, 16:8 h (day/night), for 6 wk altogether. Throughout the first week of the experiment, soil water content was maintained at 70% WHC for all three groups of plants (WW, WDS, and FC). After that, during the second week, the WDS pots were left to dry out until the soil water content reached 35% WHC; the FC pots were watered to reach 95% WHC; and in the WW pots, WHC was maintained at 70%. All three treatments were maintained under these conditions for an additional 4 wk. The application of water stress was determined as reported by Meddich et al. (2015), with minor changes.

Measurements of the stem and root length, plant mass, and biochemical analysis of the basil plant were performed at the end of the experiment.

Biochemical analysis of basil plants

One gram of basil leaves was homogenised in a cooled mortar and pestle with 10 mL phosphate buffer (0.1 M KH_2PO_4, pH 7), centrifuged for 10 min at 4500 rpm, and the supernatant was used for further biochemical analyses. All analyses were determined spectrophotometrically by a Lambda 25 UV/Vis spectrophotometer (Perkin Elmer, Waltham, Massachusetts, USA).

Total protein content was determined by the Bradford method (Bradford, 1976). Protein content was expressed as mg g^{-1} FW plant material. Lipid peroxidation intensity (intensity of membrane lipids peroxidation) was determined by Hodges et al. (1999). As the secondary product of the oxidation of polyunsaturated fatty acids, malondialdehyde (MDA) content was measured as the equivalent of lipid peroxidation intensity. Lipid peroxidation intensity in basil leaves was calculated from formulas explained in the reference method and expressed as nmol MDA equivalents g^{-1} FW. Superoxide dismutase (SOD) activity was determined by Chen and Zhang (2016). One unit of SOD is defined as the amount of enzyme that inhibits 50% nitroblue tetrazolium photoreduction, and SOD activity was expressed as U g^{-1} FW.

Glutathione (γ-L-glutamyl-L-cysteinyl-glycine) is involved in response to abiotic and biotic stress, and implicated in the primary metabolism (C, N, and S metabolism) of the cell (Noctor et al., 2012). The reduced (GSH) and oxidised forms (GSSG) of glutathione and their redox state is normally tightly controlled, but during stress conditions, the oxidised form can prevail (Tausz et al., 2004). Total reduced glutathione content was determined as total non-protein thiol compounds were expressed as GSH equivalents calculated from the GSH calibration curve and expressed as μM GSH g^{-1} FW. Total phenolic compounds were determined by Folin-Ciocalteu method (Makkar, 2003), calculated from the standard curve, and expressed as gallic acid equivalents per fresh weight of plant material (mg GAE g^{-1} FW).

Statistics

Values of the tested parameters were expressed as means ± standard error of determinations made in triplicates and tested by ANOVA followed by comparison of the means by Duncan's multiple range test and Fisher's LSD test ($P < 0.05$). These data were analysed using Statistica 13 (TIBCO, Palo Alto, California, USA).

RESULTS

Based on the morphological characteristics of cells and colonies, a total of five representative bacterial isolates were selected: Two *Azotobacter* isolates (A13, A14), one *Pseudomonas* isolate (P57), and two *Bacillus* isolates (B79, B82).

PGP characterization of the isolates

The PGP characterization of all investigated isolates is presented in Table 1. Two strains (B79 and B82) produced IAA and three isolates (B79, B82, and P57) produced siderophores. All studied isolates were positive for HCN production.

A test for ACC deaminase activity was positive for four (A13, A14, P57, B82) out of the five isolates. Only one isolate (A13) solubilized, while three isolates (A13, A14, B82) mineralized phosphate. According to the results of present study, 60% isolates were positive for lipase activity, 80% for amylase and 40% for pectinase and cellulase activity.

Table 1. Plant growth-promoting and biocontrol traits of the isolates. [1]Indol-3-acetic acid production +, – not detected. [2]Width of orange zone: – no zone, + 1-4 mm, ++ 5-20 mm, +++ ≥ 20 mm. [3]Hydrogen cyanide productions evaluated according colour and its intensity: + minimal, ++ medium, +++ large, ++++ the largest production, – not detected. [4]Effectiveness of phosphate solubilization and mineralization of P organic compounds evaluated according to zone diameter: + represents 4 mm d^{-1}, ++ represents ≥ 5 mm d^{-1}. [5]1-Aminocyclopropane-1-carboxylate deaminase activities: presence of an activity is indicated by + whereas the absence is indicated by -. [6]Lipase (L), amylase (A), pectinase (P), cellulase (C) activities: + hydrolysis; - no hydrolysis.

Isolates	IAA[1]	Siderophore[2]	Hydrogen cyanide[3]	Phosphate mineralization[4]	Phosphate solubilization[4]	ACC[5]	Enzymes[6]			
							L	A	P	C
A13	-	-	++	+	+	+	-	+	-	+
A14	-	-	++	++	-	+	++	+	-	-
P57	-	+	++++	-	-	+	++	-	+	-
B79	+	++	+++	-	-	-	-	+	-	+
B82	+	+	+++	+	-	+	+	+	+	-

Evaluation of isolates for their PGP potential on basil plants

In this study, the application of the isolates enhanced the number of germinated seeds (Table 2). After 7 d, the highest number of germinated seeds was obtained in the variant with the A13 isolate (96.0%), while the smallest number was determined in the control variant (50.0%).

The introduction of isolates had a good effect on the root and shoot length of germinated seeds (Table 2). On average, the best results were achieved with *Azotobacter* and *Bacillus* isolates. All isolates increased seedling vigour. The highest response for vigour index was observed with *Azotobacter* isolates (7200.0% and 5628.0%).

The effect of isolates application on investigated plant growth parameters is showed in Table 3. It was observed that all applied isolates positively affected stem length and plant mass under well-watered and drought-stressed conditions of cultivation. The best results were achieved for plant mass. On average, *Azotobacter* isolates A13 and A14 and *Pseudomonas* isolate P57 had the most significant influence on the plant mass. In well-watered conditions, basil mass inoculated with P57 was by 34.2% higher than in the control. In drought-stressed conditions, the plant mass inoculated with *Azotobacter* isolates was by 90% (A13) and by 80% (A14) higher than in the control. In flooded-stressed conditions, the plant mass inoculated with *Azotobacter* and *Pseudomonas* isolates increased by more than 100%.

Regarding root and stem length, isolates had different influences on their development. The best result for root length was achieved by inoculation of *Azotobacter* isolate A13 in well-watered conditions and by P57 in drought-stressed conditions. In flooded stressed conditions, root length of all variants was lower than control. In variants P57 and B79, the decrease in root length was significant. The best stem length was achieved with P57 (well-watered and flooded-stressed conditions) and A14 isolates (drought-stressed conditions).

Table 2. Effect of selected isolates on seed germination, root and shoot length and vigour index (VI). Values in the same column followed by different letters indicate significant differences ($p < 0.05$) between the means.

				Root length		Shoot length		
	Germinated seeds							
Isolates	3 d	7 d	Seed germination	7 d	10 d	7 d	10 d	VI
	Nr		%	mm		mm		%
A13	45.0^{a}	48.0^{a}	96.0	30.0^{a}	35.0^{a}	35.0^{a}	40.0^{a}	7200.0
A14	29.0^{c}	32.0^{c}	64.0	20.0^{b}	30.0^{b}	30.0^{b}	37.0^{b}	5628.0
P57	28.0^{c}	32.0^{c}	64.0	10.0^{c}	16.0^{e}	30.0^{b}	34.0^{cd}	4200.0
B79	35.0^{b}	36.0^{b}	72.0	15.0^{c}	20.0^{c}	25.0^{c}	35.0^{c}	5060.0
B82	34.0^{b}	35.0^{b}	70.0	15.0^{c}	16.0^{e}	25.0^{c}	35.5^{bc}	4635.0
Control	20.0^{d}	25.0^{d}	50.0	15.0^{c}	18.0^{d}	25.0^{c}	33.0^{d}	3978.0

Table 3. Effect of selected isolates application on stem and root length and plant mass. Values in the same row column followed by different letters indicate significant differences ($p < 0.05$) between the means.

	Well-watered			Drought-stressed			Flooded-stressed		
Isolates	Root	Stem	Mass	Root	Stem	Mass	Root	Stem	Mass
	mm		g	mm		g	mm		g
A13	20.0^{a}	39.1^{bcd}	21.4^{cd}	10.2^{b}	19.5^{b}	3.8^{a}	18.1^{ac}	36.6^{b}	37.1^{a}
A14	18.4^{ab}	40.5^{ab}	24.2^{b}	10.5^{b}	20.3^{a}	3.6^{b}	16.4^{c}	37.8^{b}	25.6^{b}
P57	16.6^{c}	40.7^{a}	26.7^{a}	10.9^{a}	17.6^{d}	2.9^{c}	13.6^{b}	41.0^{a}	24.3^{b}
B79	18.0^{b}	38.4^{cd}	22.9^{bc}	10.3^{b}	17.6^{d}	2.4^{d}	12.4^{b}	40.2^{a}	15.0^{d}
B82	18.0^{b}	39.7^{abc}	19.9^{d}	10.2^{b}	18.6^{c}	2.9^{c}	17.6^{ac}	40.0^{a}	18.7^{c}
Control	18.0^{b}	37.8^{d}	19.9^{d}	10.1^{b}	13.7^{e}	2.0^{e}	18.4^{a}	34.7^{c}	12.4^{e}

Biochemical analysis of basil plants

Protein content varied among plants that were inoculated with different microorganisms (MO) and grown under different abiotic stress treatments (AST), yet those that were grown under dry conditions had higher total proteins contents than other AST, especially plants treated with *Pseudomonas* sp. P57 isolate (12.99 mg g^{-1}) (Table 4).

The intensity of lipid peroxidation was higher (15.09-28.32 nmol g^{-1}) in plants under flood treatments (within AST and among MO treatments), except for those inoculated with *Bacillus* sp. B79 and *Pseudomonas* sp. P57 isolates, which had the highest LP intensity under drought conditions (10.92-19.00 nmol g^{-1}, respectively).

The highest SOD activity was measured in plants under flood treatments, inoculated with *Pseudomonas* sp. P57 and *Azotobacter* sp. A14 isolates, while the highest content of GSH had plants treated with *Pseudomonas* sp. P57 isolate under dry and flood conditions. Additionally, plants inoculated with *Pseudomonas* sp. P57 isolate and grown under dry conditions also had the highest total phenolics content (4.84 mg g^{-1}).

DISCUSSION

Medicinal plants harbour a distinctive microbiome due to their unique and structurally divergent bioactive secondary metabolites, which are most likely responsible for the high specificity of the associated microorganisms. Our research illustrates that rhizospheric bacteria of basil have multiple PGP traits. Obtained results show that not all the collected isolates possessed tested PGP activities, which confirmed their difference when PGP activities are concerned. This is similar to the findings of Malleswari and Bagyanarayana (2013), who reported analogous results in evaluating the PGP activities of bacterial isolates from the rhizosphere of different medicinal and aromatic plants in Andhra Pradesh. Out of 112 collected isolates, only eleven had distinguished PGP activities.

Table 4. Contents of soluble proteins (P), lipid peroxidation intensity (LP), superoxide dismutase (SOD) activity, reduced glutathione (GSH) and total phenolics (TP) contents. Values represent the mean ± standard error (SE). Results in the same column marked with different lowercase (difference among microorganisms treatments within abiotic stress treatment) and uppercase letters (difference among abiotic stress treatments within microorganisms treatment) differ significantly at $p < 0.05$ (Duncan's multiple range test).

Isolates	Conditions	P	LP	SOD	GSH	TP
		mg g^{-1}	nmol g^{-1}	U mg^{-1}	mM g^{-1}	mg g^{-1}
A13	WW	4.81 ± 0.06^{eB}	0.87 ± 0.02^{eC}	20.59 ± 0.01^{cB}	1.19 ± 0.01^{fC}	2.54 ± 0.00^{cB}
	WDS	7.59 ± 0.07^{dA}	2.39 ± 0.06^{eB}	22.39 ± 0.01^{aA}	1.90 ± 0.00^{eA}	2.83 ± 0.01^{cA}
	FC	7.60 ± 0.06^{bA}	18.22 ± 0.16^{bA}	18.11 ± 0.02^{eC}	1.71 ± 0.00^{dB}	2.35 ± 0.01^{cC}
A14	WW	5.71 ± 0.06^{dB}	16.81 ± 0.06^{aB}	21.83 ± 0.02^{bB}	2.10 ± 0.01^{abB}	2.67 ± 0.01^{bA}
	WDS	9.81 ± 0.07^{bA}	6.79 ± 0.07^{dC}	16.24 ± 0.01^{dC}	2.53 ± 0.01^{bA}	2.55 ± 0.01^{dB}
	FC	5.98 ± 0.07^{dB}	20.28 ± 0.09^{aA}	29.24 ± 0.04^{bA}	1.92 ± 0.01^{cC}	2.51 ± 0.01^{bB}
P57	WW	8.20 ± 0.06^{aB}	9.29 ± 0.07^{cB}	18.16 ± 0.35^{dB}	1.28 ± 0.01^{eC}	3.20 ± 0.01^{aB}
	WDS	12.99 ± 0.01^{aA}	19.91 ± 0.05^{aA}	11.63 ± 0.01^{eC}	4.43 ± 0.01^{aA}	4.84 ± 0.00^{aA}
	FC	5.27 ± 0.03^{eC}	9.40 ± 0.05^{eB}	36.21 ± 0.02^{aA}	2.56 ± 0.01^{aB}	2.34 ± 0.01^{cC}
B79	WW	6.21 ± 0.06^{cC}	0.83 ± 0.01^{eC}	24.05 ± 0.00^{aA}	1.33 ± 0.01^{dC}	2.34 ± 0.00^{dC}
	WDS	9.05 ± 0.03^{cA}	10.92 ± 0.06^{cA}	16.66 ± 0.02^{dC}	2.21 ± 0.01^{cA}	2.91 ± 0.01^{cA}
	FC	6.99 ± 0.07^{cB}	5.77 ± 0.09^{fB}	17.47 ± 0.02^{eB}	1.67 ± 0.01^{eB}	2.51 ± 0.00^{bB}
B82	WW	6.20 ± 0.06^{cC}	2.69 ± 0.06^{dB}	15.17 ± 0.02^{eC}	1.65 ± 0.01^{cB}	2.72 ± 0.00^{bA}
	WDS	6.82 ± 0.06^{eB}	0.37 ± 0.19^{fC}	19.79 ± 0.02^{bB}	1.52 ± 0.01^{fC}	2.29 ± 0.00^{eC}
	FC	8.25 ± 0.03^{aA}	17.30 ± 0.06^{cA}	22.41 ± 1.04^{dA}	2.23 ± 0.01^{bA}	2.53 ± 0.01^{bB}
Control	WW	7.20 ± 0.06^{bB}	14.06 ± 0.03^{bB}	19.50 ± 0.01^{cB}	1.88 ± 0.02^{bB}	3.22 ± 0.00^{aA}
	WDS	8.89 ± 0.06^{cA}	14.10 ± 0.06^{bB}	17.70 ± 0.01^{cB}	2.03 ± 0.01^{dA}	3.07 ± 0.04^{bB}
	FC	5.19 ± 0.06^{eC}	15.09 ± 0.07^{dA}	27.39 ± 0.06^{cA}	1.67 ± 0.01^{eC}	2.78 ± 0.01^{aC}

Many reports have observed the remarkable positive effects of PGPR on seed vigour index and germination rate (Jahanian et al., 2012), which aligns with the results of our research. However, some studies show that certain PGP strains can negatively affect germination rate and initial growth. According to Sehrawat et al. (2022), the inhibitory effect of the PGPR could be due to their ability to produce HCN. In contrast to these studies, the present work showed that despite the fact that the isolates produced HCN, they all had a positive impact on the seed germination rate and vigour index.

The survival of plants under adverse environmental conditions depends on their capability to integrate stress-adaptive metabolic and structural changes into endogenous metabolism (Chiappero et al., 2019). Water stress can affect biochemical activities and prompt the biosynthesis of several substrates like ethylene, restricting plant growth. Adverse effects of water stress were observed in many aromatic and medicinal plants, including *Lavandula latifolia*, *Mentha piperita*, *Salvia sclarea, Salvia lavandulifolia*, *Thymus mastichina* and *Thymus capitatus* (García-Caparrós et al., 2019). In the present study, plant growth was also significantly reduced by water stress, but it was improved by PGPR application. Similarly, many authors have reported improved medicinal plant growth by PGPR under water-stressed conditions (Verma and Saharan, 2020). The reason behind these plants' growth improvement might be the reduction of ethylene concentration due to isolates with higher ACC-deaminase activity. Ethylene is a very important phytohormone, but only at minimal concentrations. Under stress conditions (salinity, drought, waterlogging, temperature, pathogenicity, and contaminants), the content of ethylene increased quickly and caused adverse impacts on plant growth. Enzyme ACC-deaminase removes ethylene precursor, which decreases ethylene concentration, thus, low accumulation of ethylene in roots results in a significant improvement in plant development. Saleem et al. (2007) proposed that it is imperative to regulate ethylene production in the close vicinity of plant roots for the normal growth and development of the plants. Besides ACC-deaminase activity, Mohite (2013) suggested that growth hormone IAA is an allied factor, which might also be responsible for improvement in root elongation and better plant growth under water-stressed conditions. High secretion of IAA by PGPR leads to modifications in the root

morphology that can alleviate the adverse effects of stress and increase the ability to absorb nutrients and water. In Jabborova et al. (2021) study, inoculation with IAA producing PGPR significantly improved the growth attributes and photosynthetic potential of soybean under drought conditions. In our study, isolates revealed ACC-deaminase activity (except B79), and two isolates produced IAA (B79 and B82). It could explain the enhanced plant growth under water-stressed conditions. Similarly, Dodd et al. (2005) investigated the physiological responses of pea (*Pisum sativum* L.) to inoculation with ACC deaminase bacteria under moisture stress and watering conditions. This experiment observed the positive effects of ACC deaminase bacteria on root and shoot biomass, leaf area, plant transpiration, seed yield, and seed N accumulation.

Due to their contribution to signalling pathways in plant metabolism, especially during (a)biotic stress, antioxidants are important markers of plants responses to various environmental stimuli. Antioxidant enzymes (catalase-CAT, ascorbate peroxidase-APX, SOD, etc.) and nonenzymatic antioxidants (ascorbic acid, glutathione, tocopherols, and carotenoids) alleviate oxidative stress (induced by reactive oxygen species) or directly impact the growth and developmental mechanisms of plants (Singh et al., 2021).

Drought stress greatly affects basil plants photosynthetic system, decreases total phenolics, carotenoids, chlorophyl *a*, and total chlorophylls contents, enhances peroxidases activity, and increases glutathione, ascorbate, and MDA contents, especially at higher CO_2 concentrations (720 ppm) (Barickman et al., 2021).

There are a number of studies on the PGPR effect on different subspecies or cultivars of basil plants exposed to diverse abiotic stresses (Kumar et al., 2022), such as salinity stress (Yilmaz et al., 2023), drought (Heidari and Golpayegani, 2012; Mahdavikia et al., 2019), flooding (Agami et al., 2016), etc.

According to Heidari and Golpayegani (2012), a combination of *Azospirillum brasilense*, *Bacillus lentus*, and *Pseudomonades* sp., showed the highest glutathione peroxidase (GPX) and APX activity and chlorophyll content in leaves of basil plants under water stress (water regimes of 40%, 60%, and 80%, of the field capacity for treatments 1 and 2 and control, respectively), while *Pseudomonades* sp. significantly increased the CAT enzyme activity. In the current study, measured SOD activity significantly increased only under the flood regime, being the highest in treatments with *Pseudomonas* sp. P57 and *Azotobacter* sp. A14 isolates. Mahdavikia et al. (2019) confirmed that basil plants treated with a mixture of PGPR (*Azotobacter vinelandii*, *Pseudomonas putida*, *Bacillus lentus*, *Pseudomonas koreensis*, and *Pseudomonas vancouverensis*) under water limitation treatment had 1.7-1.9 times higher SOD activities than plants from the control. Similarly to the findings of Chiappero et al. (2019), who investigated the effect of inoculation with *Bacillus amyloliquefaciens* and *Pseudomonas fluorescens* isolates on *Mentha piperita* plants under drought conditions, *Bacillus* and *Pseudomonas* isolates from this study alleviated the lipid peroxidation process in inoculated plants under drought treatments, but under flood conditions their effect was lacking. The same study showed that accumulation of total phenolics enhanced 2.0- and 2.6-fold in correspondence to severity of a treatment applied (50% and 35% field capacity irrigation, respectively). However, *M. piperita* plants, in general, had lower lipid peroxidation (approx. 25-90 μmol g^{-1} FW) and phenolics content (200-900 μg GAE g^{-1} FW), as well as significantly higher SOD activity (more than 10-times) than *O. basilicum* plants in this study.

Agami et al. (2016), reported that a PGPR mixture consisting of *Azotobacter chrocoocum* A101, *Pseudomonas fluorescens*, *Pseudomonas mendocina* Palleroni 1970, and *Azospirillum lipoferum* N040 significantly alleviated water stress in basil plants by enhancing leaf water potential and endogenous production of proline, carotenoids, soluble sugars, and antioxidant enzymes (SOD, CAT, and polyphenol oxidase).

CONCLUSIONS

The results of this experiment reveal that rhizospheric bacteria of *Ocimum basilicum* L. var. *minimum* have multiple biochemical and plant growth-promoting (PGP) properties. The most intensive reaction on tested abiotic stressors - drought (higher total phenolics, reduced glutathione, and malondialdehyde content) and flooding (higher superoxide dismutase activity); were from the basil plants inoculated with *Pseudomonas* sp. P57 isolate, yet along with *Azotobacter* sp. isolate A13, these significantly improved seed germination percentage and vigour index of seedlings. Further testing of this isolate as a priming agent or a mixture of isolates of all three species, *Azotobacter* sp., *Pseudomonas* sp., and *Bacillus* sp., as a biofertilizer under field conditions could confirm their true potential. Bearing in mind the efficiency of tested PGP rhizobacteria, as well as food security, and a restriction on the use

of artificial fertilisers or a ban on pesticides in medicinal and aromatic plants and spices production, it is of crucial importance to test the application of these valuable bacterial species as a future agrotechnological practise.

Author contribution

Conceptualization: D.S, T.H.J., B.K. Methodology: D.S., B.K., S.Đ. Formal analysis: T.H.J. Investigation: D.S., B.K. Writing-original draft: D.S., B.K. Writing-review & editing: T.H.J., S.Đ., M.A. All co-authors reviewed the final version and approved the manuscript before submission.

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