Role of EPS produced by *Brevundimonas faecalis* BC1 in enhancing heat and drought stress tolerance in *Salvia hispanica* and *Vigna radiata* Microgreens

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Abstract: Drought and heat are the major abiotic factors that can cause yield loss in major agricultural crops and pose a big threat worldwide. These stresses disrupt plant homeostasis by causing oxidative damage to them and lowering water holding potential. Microgreens are very particularly vulnerable to these stresses due to their shallow root and high metabolic activity, which lead to reduce in nutritional value and total biomass. In this study, EPS from *Brevundimonas faecalis* BC1 was used as a protective agent in *Salvia hispanica* and *Vigna radiata* microgreens under drought and heat-stressed conditions. 2% EPS added to the growth media of the microgreens and five experimental setups has been prepared as control, heat stress, drought stress, EPS + heat stress, and EPS + drought stress. The bacterial EPS *from Brevundimonas faecalis* BC1 enhanced the resilience of *Salvia hispanica* and *Vigna radiata* microgreens to thermal and drought stress. The treated seedlings exhibited superior water retention, enhanced root and shoot development, and reduced stress-related damage compared to untreated plants and suggests that EPS facilitated the establishment of immature seedlings by creating a more favourable microenvironment around the roots and mitigating the adverse effects of abiotic stress. Its hygroscopic and adhesive properties may have preserved moisture and physiological stability in unfavourable situations. From this analysis, it is identified that, EPS can act as a biostimulant and can enhance drought and heat stress tolerance capability of microgreens.

Keywords: EPS, *Brevundimonas faecalis* BC1, microgreens, abiotic stress tolerance

1. Introduction:

Abiotic stresses like heat and drought are one of the major environmental factors which can affect the plant growth and need immediate attention from scientific community [1]. These stresses can disturb cellular metabolism processes, reduce the water content and generation of reactive oxygen species which can damage cell membrane, proteins and nucleic acids [2]. *Salvia hispanica* and *Vigna radiata* are two of the most cultivated plants due to their high nutritional value and considered as one of the important foods as microgreen. Microgreens are juvenile, immature plants which usually harvested during the cotyledon stage and currently they are considered as one of the major sources of nutrition in urban market and has potential commercial value. When these microgreens undergo different abiotic stresses, the growth of the plants, nutritional value get affected severely, which require the use of biostimulants to aid the growth of the plants in stressed condition [3]. Exopolymeric substances (EPS) is a mixture of biological macromolecules secreted by biofilm forming microorganisms like bacteria, fungi, cyanobacteria and algae to establish the biofilm community on a solid surface [4]. The EPS play a crucial role in establishment, protection and communication in between different species present in the biofilm community. *Brevundimonas faecalis* BC1, a gram-negative biofilm forming bacteria initially isolated from the epilithic biofilm of the monumental rocks. From previous study, it was identified that the bacteria can survive in higher temperature by secreting more EPS to the outer environment and altering its protein, polysaccharides and bioactive compound compositions, its hygroscopic and sticky nature [5]. Based on these characteristics, we hypothesized that the EPS can act as a biostimulant that holds more water, protects plants from higher temperatures, and provides essential nutrients for plant growth. So, in this study, EPS from *Brevundimonas faecalis* BC1 was mixed with the growth medium. The experimental design included drought and heat stress treatments applied to the plants, along with a control group, to evaluate plant growth rate and biochemical composition.

2. Materials and Methods:

2.1. Bacterial strain and EPS production: EPS producing bacterial strain *Brevundimonas faecalis* BC1 collected from monumental rock [5] of Kunti Temple, Mahendragiri, Odisha, India (18°57' 51" N, 84°22' 10" E) has been used in this investigation for production of EPS. Standard microbial culture methods were followed throughout the study to ensure reproducibility and consistent growth across all experiments. EPS from the bacteria has been obtained by following standard EPS extraction method [6]. The extracted EPS was freeze dried and stored for further analysis.

2.2. Selection of seeds and microgreen cultivation: Seeds of *Salvia hispanica* (Chia) and *Vigna radiata* (Moong) has been selected for cultivation of microgreens in this study. Healthy seed of *Salvia hispanica* and *Vigna radiata* has been collected and the seeds were cleaned and surface sterilized to minimize microbial contamination and sown in trays containing mist growing medium composed of cocopit and vermicompost (3:1 w/v) under controlled environmental condition. The cultivation and growth of the microgreens have been done by following standard microgreen cultivation method [7]. After seeding, the trays were placed in low light or darkness for germination (3 days) before being moved to light for cotyledon development. The microgreens were taken at the cotyledon stage (7 days after seeding) for further analysis, and moisture was maintained by frequent misting without waterlogging [8].

2.3. Experimental setup: For this experiment five different experimental setup has been followed i.e. control (30°C), heat stress (42°C), drought stress, EPS + heat stress, and EPS + drought stress. The experiment was arranged in Completely Randomized Design (CRD) in triplicate (n=3) format. Specific number of healthy and viable seedlings has been assigned to all the treatment groups. The EPS treatment was applied at a concentration of 2% (w/v) after establishment of the cultivation system and stressed condition was maintained at cotyledon stage. PEG 6000 was used to induce drought stress conditions as it can simulate osmotic water deficit. Drought stress was maintained for 4 days by applying 10% (w/v) PEG 6000 in the experimental trays. Likewise, heat stress was induced by placing the seedlings in growth chamber maintained at 42°C for 4 days. After the completion of stress period, the microgreens were harvested and used for further analysis.

2.4. Growth measurement: After harvesting the microgreens the growth parameters like root length and shoot length were measured. Fresh biomass were recorded immediately after harvest and dried biomass of the microgreens were also recorded after oven-drying the samples.

2.5. Biochemical estimations

2.5.1. Protein and carbohydrate estimation: Protein and carbohydrate estimation of the collected microgreen was done by following the Lowry protein assay [9] and phenol sulfuric acid method [10] respectively.

2.5.2. Proline content: The acid ninhydrin technique was used to determine free proline. After homogenizing fresh plant tissue in 3% sulfosalicylic acid, glacial acetic acid and acid ninhydrin were added to the filtrate. The chromophore was extracted with toluene following incubation in a boiling water bath, and absorbance was measured at 520 nm [11]. A standard curve developed with known proline concentrations was used to calculate the proline content.

2.5.3. Total phenolic content: The Folin-Ciocalteu technique was used to calculate the total phenolic content. After combining the plant extract with the Folin-Ciocalteu reagent and sodium carbonate, the reaction mixture was left to develop colour in the dark. A standard curve was used to express the results as gallic acid equivalents after absorbance was determined spectrophotometrically, typically at 760–765 nm [12].

2.5.4. Relative water content: Relative water content was estimated from fresh leaf or seedling tissue by recording fresh weight, turgid weight (after soaking in water), and dry weight (after oven drying). The conventional formula was used to determine the relative water content:

(Turgid weight−Dry weight) × 100 (Fresh weight−Dry weight)/(Turgid weight−Dry weight) × 100.

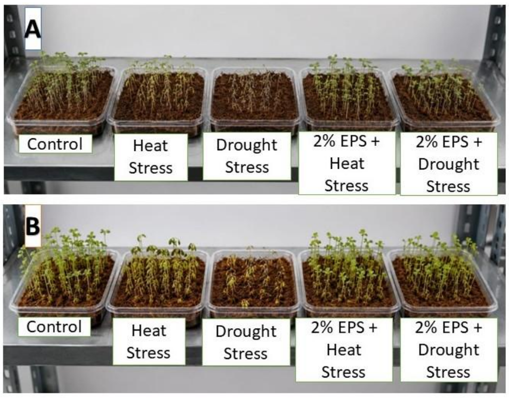
3. Results and Discussion:

3.1. Growth parameters: Growth parameters of all the experimental conditions (control, heat stress, drought stress, EPS + heat stress, and EPS + drought stress) were analyzed based on the measurements of root length, shoot length and plant height (fig.1, Table 1). Among all the experimental conditions, the control showed the highest growth compared to the heat and drought stress treatments. However, treatment with 2% (w/v), EPS resulted in significantly higher growth compared to the untreated condition. EPS-treated seedlings exhibited enhanced root and shoot growth under stress, indicating that EPS mitigated the inhibitory effects of environmental stress on early seedling establishment [13].

Table 1: Growth parameter analysis of microgreen in experimental conditions

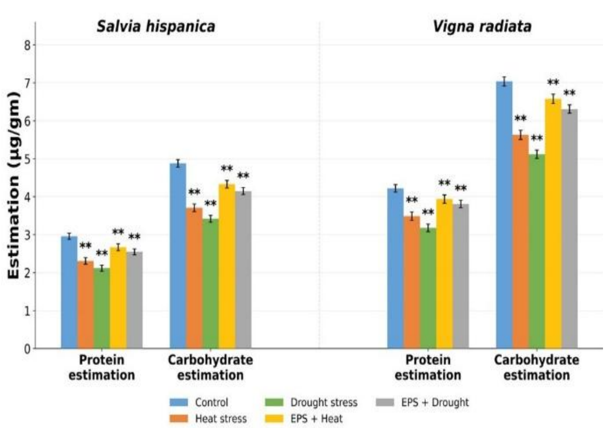
Treatment	Root length(cm)		Shoot length (cm)		Seedling length (cm)	
	Salvia hispanica	***Vigna radiata***	***Salvia hispanica***	***Vigna radiata***	***Salvia hispanica***	***Vigna radiata***
Control	2.9 ± 0.1	4.3 ± 0.2	4.8 ± 0.2	7.1 ± 0.3	7.7 ± 0.2	11.4 ± 0.4
Heat stress	2.2 ± 0.1	3.4 ± 0.1	3.6 ± 0.2	5.6 ± 0.2	5.8 ± 0.2	9.0 ± 0.3
Drought stress	2.0 ± 0.1	3.1 ± 0.1	3.3 ± 0.1	5.1 ± 0.2	5.3 ± 0.2	8.2 ± 0.3
EPS (2%) + Heat stress	2.6 ± 0.1	4.0 ± 0.2	4.3 ± 0.2	6.6 ± 0.2	6.9 ± 0.2	10.6 ± 0.4
EPS (2%) + Drought stress	2.5 ± 0.1	3.8 ± 0.1	4.1 ± 0.2	6.3 ± 0.2	6.6 ± 0.2	10.1 ± 0.3

Fig.1. Effect of heat and drought stress on the growth of *Salvia hispanica* (A) and *Vigna radiata* (B) microgreens grown in cocopit under different experimental conditions with EPS application (2%, w/v).



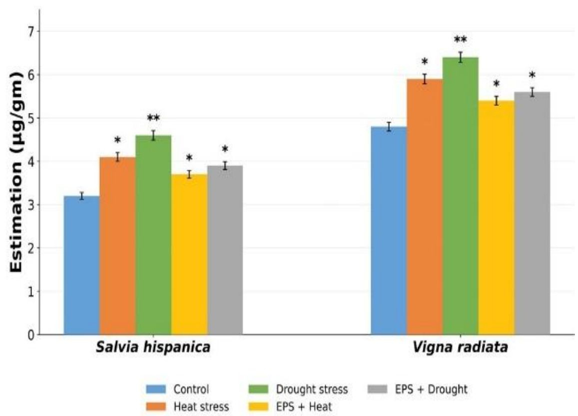
3.2. Biochemical analysis: In *Salvia hispanica*, protein content decreased from 2.96 ± 0.08 in the control to 2.31 ± 0.07 under heat stress and 2.12 ± 0.06 under drought stress. A similar trend was observed in *Vigna radiata*, where protein content declined from 4.22 ± 0.11 in the control to 3.49 ± 0.10 under heat stress and 3.18 ± 0.09 under drought stress. This reduction suggests that both stresses probably affected nitrogen metabolism and protein synthesis which is common under abiotic stress, as such stress downregulates energy metabolism and protein production and channels resources toward survival pathways. The EPS-treated plants showed improved protein content under stress, with values rising to 2.67 ± 0.07 in *Salvia hispanica* and 3.94 ± 0.10 in *Vigna radiata* under heat stress, and 2.55 ± 0.07 and 3.81 ± 0.09 under drought stress, respectively. This result corroborates with the findings that bacterial EPS can enhance plant tolerance to heat and drought stress by stabilizing membrane function and promoting stress resilience [14]. In *Salvia hispanica*, carbohydrate content declined from 4.88 ± 0.10 in the control to 3.71 ± 0.09 under heat stress and 3.42 ± 0.08 under drought stress. In *Vigna radiata*, the decrease was from 7.04 ± 0.16 in the control to 5.63 ± 0.14 and 5.12 ± 0.13 under heat and drought stress, respectively (fig.2). The reduction in carbohydrate content under stress may be due to impaired photosynthesis, altered sugar metabolism, and increased use of carbohydrates for stress defense, as abiotic stress often suppresses carbon fixation while changing carbohydrate partitioning and osmotic adjustment [15]. However, EPS treatment improved carbohydrate levels in both crops, indicating better maintenance of photosynthetic capacity and energy balance during stress, which aligns with the protective role of microbial EPS in improving water retention and physiological stability under adverse conditions [13]

Fig.2. Effect of heat stress, drought stress, EPS (2%) + heat stress, and EPS (2%) + drought stress on protein and carbohydrate estimation in *Salvia hispanica* and *Vigna radiata*. Data are represented as mean±SEM (n=3), ** indicates p < 0.01 compared with the control.



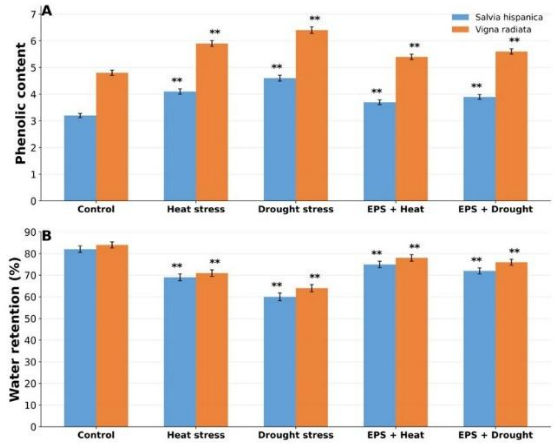
In this experiment it was also observed that proline content (µmol g^{-1} FW) increased significantly under both heat and drought stress in *Salvia hispanica* and *Vigna radiata* compared to the control. In *Salvia hispanica*, proline content increased from 3.20 ± 0.10 in the control to 4.10 ± 0.12 under heat stress and 4.60 ± 0.13 under drought stress (fig.3). In *Vigna radiata*, the corresponding increase ranged from 4.80 ± 0.14 in the control to 5.90 ± 0.16 under heat stress and 6.40 ± 0.17 under drought stress. This indicates that drought stress caused a greater accumulation of proline than heat stress, reflecting a stronger osmotic disturbance in the seedlings. Proline is a well-known compatible solute that protects cells by maintaining osmotic balance, stabilizing proteins and membranes, and scavenging reactive oxygen species under stress conditions. In EPS-treated plants, proline content was lower than in untreated stressed plants, with values of 3.70 ± 0.11 and 3.90 ± 0.11 in *Salvia hispanica* and 5.40 ± 0.15 and 5.60 ± 0.15 in *Vigna radiata* under heat and drought stress, respectively. This reduction suggests that 2% (w/v) EPS alleviated stress severity and improved physiological stability, thereby reducing the need for excessive proline accumulation.

Fig.3. Effect of heat stress, drought stress, EPS (2%) + heat stress, and EPS (2%) + drought stress on proline content in *Salvia hispanica* and *Vigna radiata.* Data are represented as mean±SEM (n=3), * indicates $p < 0.05$ and ** indicates $p < 0.01$ compared with the control.



Along with proline content, increased phenolic contents were observed in both *Salvia hispanica* and *Vigna radiata* under heat and drought stress suggesting activation of antioxidant defense mechanisms. Heat stress had a lesser effect than drought stress and the EPS treatment partially reduced the stress-induced rise. Water retention also decreased under stress, but improved after EPS treatment indicating increased physiological adjustment and stress tolerance (fig.4).

Fig.4. Effect of heat stress, drought stress, EPS (2%) + heat stress, and EPS (2%) + drought stress on phenolic content and water retention (%) in *Salvia hispanica* and *Vigna radiata.* Data are represented as mean±SEM (n=3), ** indicates $p < 0.01$ compared with the control.



The rise in industrial activity, population expansion, urbanization, deforestation, and changes in climatic conditions are significant concerns as they greatly impact agricultural activities. The development of environmental stress-resistant plants and growth factors is essential to safeguard agricultural crops against degradation which requires immediate attention from the scientific community. Microgreens are tender immature green plants harvested at the cotyledon stage, and are currently recognized as a rich source of vitamins, minerals, antioxidants, and diverse bioactive compounds. Due to their short growth cycle, small space requirement, low maintenance, and suitability for controlled cultivation, microgreens have gained increasing attention in sustainable horticulture and urban food systems. However, the microgreens highly sensitive to the environmental conditions despite their nutritional importance, because they are harvested at a juvenile stage when seedlings are physiologically delicate [16]. Microbial biostimulants have emerged as a promising tool for protecting these juvenile plants from different environmental stresses. EPS (Exopoymeric substances), a natural polymer mainly secreted by biofilm forming microorganism, which act as a protective slime layer and the main structural component of biofilm [17]. Due to the sticky and hygroscopic nature of EPS it has the ability to hold moisture [5] and as the EPS composed of complex high molecular weight polymers, it can support the growth of the microgreens. In the current study EPS was obtained from a Gram-negative bacterium *Brevundimonas faecalis* BC1, isolated from the biofilm formed on the surface of stone monument [5]. From previous study it was identified that it is majorly composed of proteins of 100, 69, 60, 52, 48, 42, and 27 kDa, polysaccharides having glucose, galactose, rhamnose and disaccharides (sucrose) in their composition and lipids as the structural components. Additionally, GC-MS analysis identified that the EPS contains different bioactive compounds, such as 2,2-dimethoxybutane and β-D-glucopyranose iduronic acid, which have potential antimicrobial activities [18]. The EPS has been used as a biostimulant for the enhancement of stress and heat tolerance in Chia and Moong microgreens. The current study revealed that drought stress and heat stress negatively impacted the growth of microgreens of *Salvia hispanica* and *Vigna radiata*. Treatment with 2% (w/v) EPS improved root length, shoot length and overall growth under stressed condition, indicating the proactive role of EPS in abiotic stressed conditions. Under stress conditions, protein and carbohydrate levels were reduced, whereas EPS-treated seedlings exhibited enhanced metabolic activity and energy balance. Elevated proline and phenolic concentrations under stress signify the initiation of defence mechanisms, but their reduced accumulation in EPS-treated plants suggested a reduction in stress severity and promoted healthy microgreen development in adverse environmental conditions.

4. Conclusion:

The current study identified that changes in environmental conditions can affect agricultural activity specifically cultivation of microgreens which are currently considered as one of the major nutritional sources. Conditions like drought and high temperature can significantly affect the physiological and biochemical properties of microgreens of *Salvia hispanica* and *Vigna radiata*. Both the stresses increased phenolic accumulation and decreased water retention, which describe the defensive nature of the microgreens. EPS of *Brevundimonas faecalis* BC1, which composed of protein, polysaccharides and bioactive compounds can act as a biostimulant and protect plants from environmental stresses like drought and increase in temperature. Treatment with 2% (w/v) EPS can significantly aid the growth of plants and protect against induced environmental stresses. However, further analysis like alterations in antioxidant activity, phytochemical profiling and correlation, stress marker analysis, dose response to better understand the mechanism of biostimulants like EPS for protection of juvenile plants like microgreens from environmental stresses.

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