



## RESEARCH ARTICLE

# Synergistic Effects of Plant-Growth-Promoting Rhizobacterium and Growth-Regulating Small Molecules on Drought Mitigation and Yield Sustainability of Rice

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## ABSTRACT

Plant-growth promoting rhizobacteria are considered as eco-friendly and sustainable inputs for providing nutrients and to ameliorate the crops from abiotic and biotic stresses. The present work was aimed to assess the stimulatory effects of plant growth-promoting rhizobacteria in association with plant-growth regulating hormonal small molecules on drought mitigation and yield of rice. The multifunctional PGPR strain *Pseudomonas chlororaphis* (ZSB15) was inoculated in rice (cultivar Co51) and assessed its synergistic impacts with three different plant-growth regulating hormonal small molecules under field conditions. The cell extracts of *Saccharomyces cerevisiae*, cell extract of *Corynebacterium glutamicum*, salicylic acid were sprayed on the foliage of rice plant under water-sufficient and water-deficit conditions. Application of cell extracts of *Corynebacteria* as a foliar spray at the seedling stage and the panicle initiation stage significantly combat the drought-induced physiological stresses of rice. It reduced the mean canopy temperature (10%), catalase activity (21%) and proline content (13.6%) of rice compared to water-sprayed control under water-deficit condition. The spray of the cell extract of *Corynebacteria* also improved the relative water content (15%) of the rice as compared to control under water-deficit conditions. It also improved the yield attributes and yield of rice both under water-sufficient and water-deficit conditions. The cell extract of yeast and salicylic acid are at par performance and relatively lower than *Corynebacterial* cell extract in terms of drought mitigation and synergistic impacts with PGPR in rice. Hence, combining the plant-growth-promoting rhizobacteria and plant-growth regulating hormonal small molecules could be a sustainable and profitable approach to increase the drought tolerance in rice to mitigate the yield losses.

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## INTRODUCTION

Drought stress is the most destructive among abiotic stresses that increased in frequency and intensity over the past decades, affecting the world's food security. It is estimated that drought stress may cause a nearly 50% loss in agricultural productivity (Kim *et al.*, 2020). Drought affects the plant's water potential and turgor, enough to interfere with normal functions, changing physiological and morphological traits in plants (Vurukonda *et al.*, 2016). It also induces secondary stress e.g. oxidative stress that can cause damage to proteins, lipids and nucleic acid in plants (Nair *et al.*, 2008). Rice is the second most important staple crop across the world having high caloric value. Rice cultivation is highly dependent on the availability of water, and drought during the sensitive flowering stage severely

affects grain yield (Ramegowda *et al.*, 2014). Several strategies are there to manage drought stress by developing drought-tolerant varieties, shifting the crop calendars, etc., (Venkateswarlu and Shanker, 2009). However, these methods are not economical and time-consuming. Recent studies postulate that plant growth-promoting rhizobacteria (PGPR) in association with plant growth regulators can help plants to cope with drought stress in a much better way (Khan and Bano, 2019; Khan *et al.*, 2019).

PGPR are the group of bacteria that inhabiting the rhizosphere and improves plant growth and development (Etesami and Maheshwari, 2018). When the PGPR are inoculated into the soil, they enhance the plant's tolerance to water deficit, hasten nutrient uptake, and increase soil moisture content (Etesami and Beattie, 2017). Plant growth

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regulators play a vital role in plant developmental processes (Asgher *et al.*, 2015). Salicylic acid (SA), a monohydroxybenzoic acid involved in cell osmotic water uptake, maintenance of cell turgor pressure, and regulation of stomatal opening (Hafez *et al.*, 2019). Cell extracts of *Corynebacterium glutamicum* and *Saccharomyces cerevisiae* are a potential source for growth factors and the amino acids in it play a role in the regulation of plant growth and development by triggering a response to various stresses (Hammad and Ali, 2014; Wendisch *et al.*, 2016). The small molecules responsible for modulating the plant growth along with PGPR strains had a significant synergistic impact in terms of yield increase and abiotic stress mitigation of various crops (Khan *et al.*, 2017; Khan *et al.*, 2018a; Khan *et al.*, 2018b; Khan and Bano, 2019; Khan *et al.*, 2019). In the present study, we evaluated the role of introduced PGPR in the growth and yield of rice plants, as influenced by the exogenous application of plant growth regulating metabolites under normal and water-deficit conditions.

## MATERIAL AND METHODS

### Plant material and Experimental site

Rice (*Oryza sativa*) short-duration variety, CO51 was used in this study. The experiment was conducted at Wetland farm, Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India during January-May, 2019. The Soil at the experimental site was clay loam having pH 8.2, electrical conductivity 0.46 dS/m, available nitrogen 265 kg/ha, available phosphorus 20.5 kg/ha and available potassium 482 kg/ha. The seeds were treated with PGPR strain *Pseudomonas chlororaphis* (ZSB15) having  $10^9$  cfu/ml and sown in a nursery (125 ml/ha seeds). After fourteen days, the rice seedlings were taken from nursery and treated with ZSB15 (250 ml per ha nursery) by root dipping method at  $10^9$  cfu/ml and then transplanted as one seedling per hill. The ZSB15 was also applied as soil application at the time of transplantation by mixed with vermicompost at  $10^9$  cfu/g (500 ml per ha).

The rice crop was cultivated by adopting system of rice intensification (SRI) method with all agronomic management practices. The recommended dose of chemical fertilizer for rice crop was  $120 \text{ kg ha}^{-1}$  nitrogen (which was applied in three split doses of urea),  $60 \text{ kg ha}^{-1}$  phosphorus in the form of single super phosphate and  $60 \text{ kg ha}^{-1}$  potassium in the form of muriate of potash. Half of the recommended dose of N along with full dose of P and K fertilizers was applied as basal (at the time of transplanting). The remaining half of N was applied as urea in two split doses on 25 and 50 days old crop as top dressing.

### Experimental Layout

The experiment was conducted based on a factorial randomized block design with five replications. Soil moisture treatments were considered as a factor – 1 and the exogenous application of small molecules treatments were considered as factor – 2. The area of the main plot was  $400 \text{ m}^2$  in which plants were grown under favorable water conditions with supplementary surface irrigation for the normal moisture condition (designated as normal). For water deficit conditions, water was drained and irrigation was withheld at 25 days after transplanting to reduce the soil moisture to 50 %. Soil moisture meter was used to determine the soil moisture content. Re-irrigation was done periodically when soil moisture fell below 50 % (designated as water-deficit). All the plants were randomly assigned to the 16  $\text{m}^2$  subplots with a spacing of 25 cm planting distance within the row.

The treatments of factor- 2 include 1) cell extract of *Corynebacterium glutamicum* (CGCE) as 0.2% spray; 2) cell extract of *Saccharomyces cerevisiae* (SCCE) as 0.2% spray; 3) salicylic acid (1 mM) spray; 4) water as control. All these hormonal small molecules were sprayed on the foliage of rice at 25 days old crop. The second round of spray was done at the panicle initiation stage.

### Observations

The chlorophyll content of leaves was measured by chlorophyll meter (Spad-502 plus, Japan) at 1) 25-days old rice plant just before an exogenous spray of small molecules (designated as B/S); 2) active tillering stage (AT); 3) panicle initiation stage (PI); 4) maturity stage just before harvest (M). The drought-mitigating traits viz., relative water content (Baldoni *et al.*, 2013), proline (Bates *et al.*, 1973) and catalase (Chandlee and Scandalios, 1984) of the leaf samples were measured following standard procedures in all the four stages. The canopy temperature was measured by an infrared thermometer at all the four stages of rice growth. The growth attributes of rice including plant height, number of tillers and panicle length were recorded at the panicle initiation stage. At the time of harvest, the yield attributes including grains per panicle, 1000-grain weight, grain yield, straw yield and harvest index (ratio between grain and biological yield) were recorded.

### Statistical analysis

All the data were subjected to analysis of variance and Duncan's multiple range test at 5% significance level using XLSTAT (version 2010.5.05) addin with Microsoft Excel for Windows 2007 to reveal the significant statistical difference among the treatments. Principal component analysis (PCA)

was performed for the observed variables of rice to reveal the similarities and differences between samples and to evaluate the relationship between exogenous chemical spray and variables (Wold et al., 1987).

## RESULTS AND DISCUSSION

In the recent past, rhizosphere engineering is considered as a powerful tool to maintain

sustainable agricultural production in unpredicted climate changes. The rhizosphere engineering can offer the plant to derive its nutrients from natural soil resources even under nutrient-limited conditions; it can provide biotic and abiotic stress mitigation, especially the drought; and also can improve the soil carbon stock (Adl, 2016). Drought is the most predominant abiotic stress being increased every year and affects crop productivity (Anjum et al., 2017).

**Table 1. Effect of exogenous application of plant regulating metabolites with PGPR on growth attributes of rice (variety Co51) under different moisture conditions**

Moisture condition	Foliar spray	Growth attributes			
		No. of tillers/plant	Panicle length (cm)	Plant height at harvest (cm)	Dry matter production at harvest (g/plant)
Normal	CGCE	31 ( 0.58) <sup>a</sup>	20.98 ( 0.23) <sup>a</sup>	125.50 ( 0.12) <sup>a</sup>	63.24 ( 0.29) <sup>a</sup>
	SCCE	30 ( 0.32) <sup>a</sup>	19.00 ( 0.05) <sup>b</sup>	122.32 ( 0.30) <sup>b</sup>	59.46 ( 0.29) <sup>b</sup>
	SA	27 ( 0.40) <sup>b</sup>	18.50 ( 0.05) <sup>c</sup>	118.30 ( 0.23) <sup>c</sup>	55.34 ( 0.32) <sup>c</sup>
	Control	25 ( 0.32) <sup>c</sup>	16.98 ( 0.09) <sup>d</sup>	113.12 ( 0.24) <sup>d</sup>	52.58 ( 0.20) <sup>d</sup>
Water-deficit	CGCE	29 ( 0.37) <sup>a</sup>	17.48 ( 0.14) <sup>a</sup>	114.38 ( 0.18) <sup>a</sup>	57.64 ( 0.74) <sup>a</sup>
	SCCE	26 ( 0.37) <sup>b</sup>	16.00 ( 0.16) <sup>b</sup>	110.46 ( 0.34) <sup>b</sup>	56.00 ( 0.19) <sup>b</sup>
	SA	26 ( 0.37) <sup>b</sup>	14.98 ( 0.06) <sup>c</sup>	106.36 ( 0.27) <sup>c</sup>	52.12 ( 0.38) <sup>c</sup>
	Control	22 ( 0.51) <sup>c</sup>	14.48 ( 0.02) <sup>d</sup>	102.80 ( 0.34) <sup>d</sup>	49.76 ( 0.21) <sup>d</sup>
CD (0.05)	T	0.80	0.25	0.56	0.76
	M	0.57	0.18	0.39	0.54
	T X M	1.13	0.36	0.79	1.07

Values are mean  $\pm$  SE (n=5) and values followed by the same letters within each column are not significantly different from each other according to DMRT ( $p \leq 0.05$ ). CGCE - *Corynebacterium glutamicum* sonicated cell extract (0.2%); SCCE - *Saccharomyces cerevisiae* sonicated cell extract (0.2%); SA - salicylic acid (1mM); Control - Water. T – exogenous chemical spray treatments; M – Moisture levels.

Based on forecasted changes in rainfall and temperature on a global scale, the drought would be an increasing challenge for agricultural production (Ings et al., 2013; Quinn et al., 2015). The plant microbiome plays a crucial role in improving the nutrient availability and drought tolerance of the crop plants under water stress conditions. The

microorganisms offer numerous ways for the drought mitigation and growth of the host plants (Vimal et al., 2017). Manipulating the plant-microbe interaction through rhizosphere engineering can enhance the performance of the host plant for agricultural production as well as for climate change mitigation.

**Table 2. Effect of exogenous application of plant regulating metabolites with PGPR on yield attributes and yield of rice (variety Co51) under different moisture conditions.**

Moisture condition	Foliar spray	Yield attributes and yield				
		Grains per panicle	1000 grain weight (g)	Straw yield (t/ha)	Grain yield (t/ha)	Harvest index
Normal	CGCE	86 ( 0.49) <sup>a</sup>	16.4 ( 0.02) <sup>a</sup>	17.4 ( 0.18) <sup>b</sup>	7.0 ( 0.07) <sup>a</sup>	31.6 ( 0.33) <sup>a</sup>
	SCCE	77 ( 0.55) <sup>b</sup>	15.6 ( 0.17) <sup>b</sup>	17.0 ( 0.17) <sup>bc</sup>	6.5 ( 0.01) <sup>b</sup>	27.7 ( 0.19) <sup>b</sup>
	SA	72 ( 0.73) <sup>c</sup>	15.2 ( 0.01) <sup>c</sup>	18.2 ( 0.04) <sup>a</sup>	5.7 ( 0.07) <sup>c</sup>	23.7 ( 0.18) <sup>c</sup>
	Control	62 ( 0.37) <sup>d</sup>	14.7 ( 0.11) <sup>d</sup>	16.9 ( 0.10) <sup>c</sup>	5.3 ( 0.03) <sup>d</sup>	23.7 ( 0.16) <sup>c</sup>
Water-deficit	CGCE	79 ( 0.40) <sup>a</sup>	15.3 ( 0.18) <sup>a</sup>	16.2 ( 0.14) <sup>a</sup>	5.0 ( 0.03) <sup>a</sup>	23.7 ( 0.15) <sup>a</sup>
	SCCE	69 ( 0.51) <sup>b</sup>	15.0 ( 0.09) <sup>a</sup>	15.7 ( 0.08) <sup>b</sup>	4.8 ( 0.03) <sup>b</sup>	23.3 ( 0.08) <sup>a</sup>
	SA	62 ( 0.37) <sup>c</sup>	15.0 ( 0.10) <sup>a</sup>	15.9 ( 0.20) <sup>ab</sup>	4.3 ( 0.00) <sup>c</sup>	21.3 ( 0.20) <sup>b</sup>
	Control	55 ( 0.68) <sup>d</sup>	14.6 ( 0.18) <sup>b</sup>	14.7 ( 0.12) <sup>c</sup>	3.4 ( 0.01) <sup>d</sup>	18.9 ( 0.17) <sup>c</sup>
CD (0.05)	T	1.11	0.27	0.26	0.08	0.37
	M	0.78	0.19	0.18	0.06	0.26
	TXM	1.57	0.38	0.37	0.11	0.52

Values are mean  $\pm$  SE (n=5) and values followed by the same letters within each column are not significantly different from each other according to DMRT ( $p \leq 0.05$ ). CGCE - *Corynebacterium glutamicum* sonicated cell extract (0.2%); SCCE - *Saccharomyces cerevisiae* sonicated cell extract (0.2%); SA - salicylic acid (1mM); Control - Water. T – exogenous chemical spray treatments; M – moisture levels

These modifications modulate the plant to improve different physiological traits including water and nutrient uptake and use efficiency, drought

tolerance, photosynthesis, growth and yield (Mendes et al., 2013; Ulrich et al., 2019). In the present investigation, we attempted a unique approach for

the first time to improve the plant-PGPR interaction via rhizosphere engineering without modifying the genome of the host plant and PGPR strain. We used the small molecules that can regulate the plant's functions to improve the root exudation, which in turn will allow the PGPR strain to colonize profusely and function effectively in the rhizosphere. This approach

has several advantages compared to genetic improvement. We showed that the application of cell extract of yeast or *Corynebacteria* or salicylic acid improved the rhizosphere colonization of soil inoculated PGPR strain (ZSB15) which in turn improved the rice growth, nutrient uptake, yield, and drought mitigation.

**Table 3. Loading values and percent contribution of assessed variables of rice under field conditions as influenced by small molecules' spray on the axis identified by the principal component analysis**

Variables	Principal component 1		Principal component 2	
	Factor loading	Contribution of variables (%)	Factor loading	Contribution of variables (%)
Panicle length (cm)	<b>0.98</b>	7.87	0.08	0.69
Plant height (cm)	<b>0.98</b>	7.87	0.10	1.09
Tillers per plant (No.)	<b>0.94</b>	7.27	-0.17	3.33
Dry matter (g/plant)	<b>0.94</b>	7.26	-0.31	11.58
SPAD index	<b>0.89</b>	6.53	0.24	6.49
Relative water content (%)	<b>0.92</b>	6.92	-0.01	0.01
Canopy temperature (°C)	<b>-0.97</b>	7.67	0.11	1.40
Proline (g per g)	<b>-0.98</b>	7.85	-0.12	1.80
Catalase (U per min per g)	<b>-0.91</b>	6.70	-0.30	10.74
Grains per plant (No.)	<b>0.95</b>	7.30	-0.26	7.90
1000 grain weight (g)	<b>0.92</b>	6.85	-0.31	11.13
Straw yield (t/ha)	<b>0.79</b>	5.14	0.59	41.36
Grain yield (t/ha)	<b>0.96</b>	7.54	-0.01	0.02
Harvest index	<b>0.94</b>	7.22	-0.15	2.48
Eigenvalue		12.25		0.86
Variability (%)		87.47		6.11
Cumulative %		87.47		93.57

Values in bold indicate a significant contribution to the corresponding principal component (>0.80)

### **Growth attributes, relative water content and Drought-responsive attributes of rice**

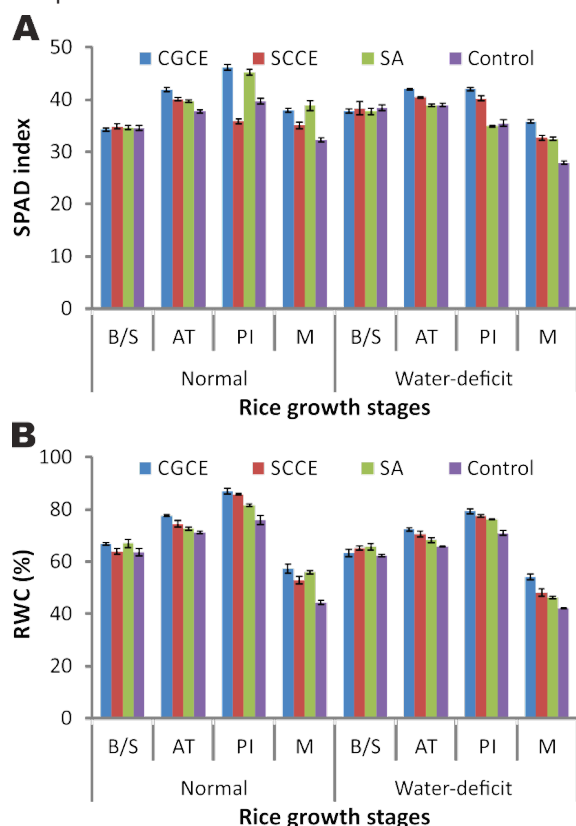
The foliar application of plant growth regulating metabolites at tillering and panicle initiation stages can increase the overall growth and yield in CO51 rice variety under both normal and 50 % moisture deficit conditions (Table 1). Significant differences were found in the number of tillers, panicle length, plant height and dry matter production between CGCE, SCCE, SA and water treatment in both normal and 50 % moisture deficit conditions. In general, all the growth attributes were higher in rice plants grown under normal moisture conditions than at water deficit conditions. Among the exogenous hormonal chemicals, CGCE spray showed a significant increase in growth attributes viz., tiller production, plant height, panicle length and dry matter production of rice, followed by SCCE and SA. The least response was reported in control plants.

The chlorophyll content (SPAD index) was increased significantly in all the treatments compared to the control at different growth stages of rice under normal and water deficit conditions after exogenous application of plant growth regulating metabolites (Fig. 1A). The chlorophyll

content was observed more in CGCE treated rice at all growth stages under both moisture conditions followed by SCCE in all growth stages at water deficit condition. Under normal moisture conditions, SA treatment showed significantly higher chlorophyll content than SCCE in the panicle initiation and maturity stage (Fig. 1A). Chlorophyll content present in normal moisture conditions was significantly higher than the moisture deficit condition. The relative water content was significantly higher in CGCE sprayed rice plant than SCCE, SA and water at different growth stages of plants, grown under normal and water deficit conditions (Fig. 1B). Relative water content was more in normal condition compared to the moisture deficit condition. The relative water content was observed more in the panicle initiation stage than the active tillering and maturity stage. The canopy temperature of rice plants during the experimental period ranged from 26°C to 38°C. The temperature is incremental with growth stages of the crop and had less impact due to moisture conditions. However, due to the exogenous spray of small molecules, the canopy temperature got reduced in the rice plant, which was noticed significantly under water-deficit conditions (Fig. 2A). The CGCE-treated plant got 2.0 to 2.5°C



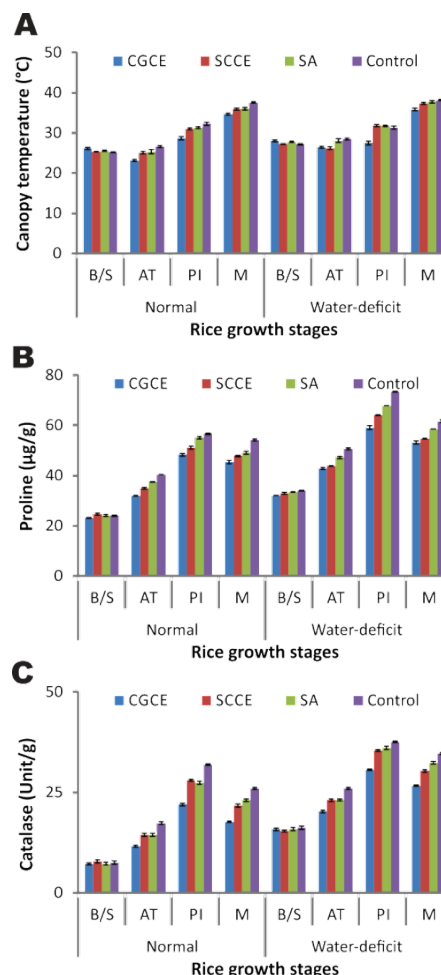
detrimental during active tillering and panicle initiation stages of rice compared to water-sprayed control plants. SCCE-treated rice also received 1.5 to 2°C reduction during the same stages of rice, while SA had an insignificant reduction in canopy temperature.



**Figure 1. Chlorophyll content as SPAD index (A) and relative water content (B) at different growth stages of rice (variety Co51) inoculated with PGPR strain, ZSB15 and sprayed with small molecules.** B/S – 25-days old rice plant before the exogenous spraying of small molecules; AT – active tillering stage; PI – panicle initiation stage; M – maturity stage. Means of five replicate values were plotted and error bars indicate the standard error. CGCE - sonicated cell extract of *Corynebacterium glutamicum* (0.2%); SCCE - sonicated cell extract of *Saccharomyces cerevisiae* (0.2%); SA - salicylic acid (1 mM). RWC – relative water content.

Leaf proline content showed significant differences among treatments at different growth stages of plants in normal and at water deficit conditions after exogenous application of plant growth regulating metabolites (Fig. 2B). CGCE treatment significantly reduced the leaf proline followed by SCCE, SA and water spray under both conditions. Proline content was observed more in moisture deficit condition compared to normal moisture condition, and more at panicle initiation stage than active tillering and maturity stage. Leaf catalase activity showed significant differences among treatments (Fig. 2C). Catalase activity was significantly reduced in CGCE treatment at all growth stages under both moisture conditions as compared to other foliar treatments. Catalase activity in SCCE and SA treatment was at par at active tillering and

panicle initiation stage of both moisture conditions, which were significantly lower than control. At the maturity stage, control showed significantly higher catalase activity than CGCE, SCCE and SA treatments. Catalase activity was significantly higher in rice under water deficit conditions than normal.



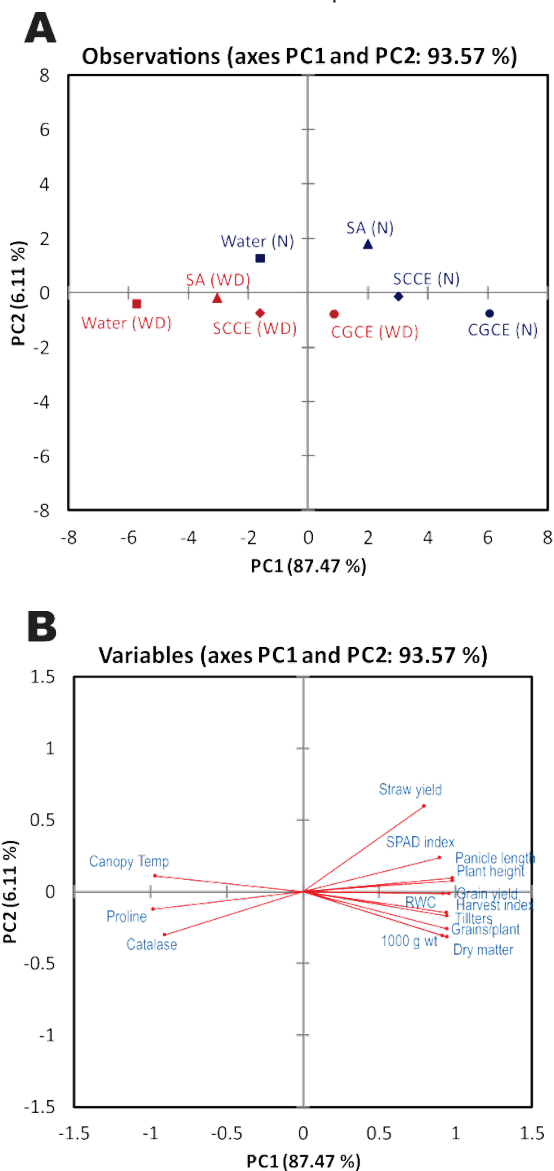
**Figure 2. Impact of exogenous spraying of small molecules and PGPR inoculation on drought-responsive attributes of rice (variety Co51) at different growth stages.**

A – canopy temperature; B – leaf proline content; C – leaf catalase activity. B/S – 25-days old rice plant before the exogenous spraying of small molecules; AT – active tillering stage; PI – panicle initiation stage; M – maturity stage. Means of five replicate values were plotted and error bars indicate the standard error. CGCE - sonicated cell extract of *Corynebacterium glutamicum* (0.2%); SCCE - sonicated cell extract of *Saccharomyces cerevisiae* (0.2%); SA - salicylic acid (1 mM).

Exogenous application of salicylic acid is a widely adopted practice for inducing defense systems against pathogens, insect pests along with growth regulation and drought and salinity resistance (Raskin, 1992). Interestingly, exogenous application of salicylic acid had a synergistic effect too with PGPR strains in terms of growth, yield and biotic and abiotic stress mitigation of several crops. Seed inoculation of *Planomicrobium chinense* (strain P1) and *Bacillus cereus* (strain P2) along with the foliar application of salicylic acid triggered the drought

tolerance of sunflower. The hormonal spray and PGPR inoculation improved the chlorophyll, protein, phenol contents of the plant along with modulation of drought mitigating physiological responsive traits viz., peroxidases and catalase activity and proline and malondialdehyde contents (Khan *et al.*, 2018b). The same strains of PGPR in combination with a foliar spray of either salicylic acid or putrescine lowered the drought-stress induced biochemical responses (proline, antioxidant enzymes, and lipid peroxidation) and ensure the yield of wheat grown in sandy soils (Khan *et al.*, 2017; Khan and Bano, 2019; Khan *et al.*, 2019). Likewise, (Hafez *et al.*, 2019) also concluded that salicylic acid in combination with PGPR strains (*Azospirillum brasilense* SARS 1001 and *Azotobacter chroococcum* SARS 302) reduced the drought-stress characters of wheat such as proline and enhanced the drought-mitigating traits such as available soil moisture, SPAD index, stomatal conductance along with yield attributes and yield under water deficit conditions. Likewise, three PGPR strains of Bacilli (*Bacillus subtilis*, *Bacillus thuringiensis*, and *Bacillus megaterium*) with salicylic acid or putrescine spray enhanced the drought tolerance of chickpea (Khan *et al.*, 2018a). Here also these combinations (PGPR and plant growth regulator) reduced the proline and stress-induced enzymes. However, in all these reports, the drought mitigating traits, plant-growth attributes and yield of host plant due to synergistic effects of various PGPR strains with foliar application of salicylic acid were elaborated. The mechanism by which these synergistic effects are being occurred in the host plant needs further investigation. A detailed transcriptomic and metabolomic analyses are needed to reveal the molecular talk among the tripartite interaction between small-molecule – host plant – PGPR. Nevertheless, in the present work, we show that exogenous application of hormonal small molecules (SA, CGCE, and SCCE) along with rhizosphere colonizing PGPR strain (ZSB15) improved the growth, yield and drought mitigation of rice. We have assessed the interaction effects under normal and water deficit conditions in rice. Irrespective of the moisture levels, the foliar application of cell extracts of yeast or *Corynebacteria* or salicylic acid with PGPR can enhance the plant growth and nutrient uptake of rice. This might be due to that foliar application of hormonal small molecules (SA, CGCE and SCCE) improves the rhizosphere functioning i.e., enhanced root exudation and subsequently the improved biological activities of the rhizosphere boosted PGPR colonization and nutrient providing the capability of the rhizosphere (Bowya and Balachandar, 2020). By all these cumulative activities, the host plant uptakes a higher amount of nutrients and this eventually reflected in the growth attributes. In the previous report, we show

that CGCE, SCCE and SA application through foliage and PGPR inoculation through root enhanced the rice constituents viz., total nitrogen, total phosphorus, total protein and total chlorophyll (Bowya and Balachandar, 2020). The results of the present work suggest that exogenous application of small molecules, apart from their regular task on plant health and fitness, can also modulate the rhizosphere functioning and thereby enhance the PGPR-mediated benefits to the host plant.



**Figure 3. Principal component analysis plots relating the observed variables on rice (Co51) under field conditions with an exogenous spray of small molecules.**

(A) Scoring plot showing the positions of treated rice plants; (B) Loading plot showing orthogonal positions of assessed rice variables. Data (n=5) collected at the time of harvest of rice plants after the exogenous spray of small molecules were used for PCA. The % variance explained by each component (PC1 and PC2) is given in parentheses in axes. In the scoring plot, (N) indicates normal moisture condition and (WD) indicates the water-deficit condition. CGCE - sonicated cell extract of *Corynebacterium glutamicum* (0.2%); SCCE - sonicated cell extract of *Saccharomyces cerevisiae* (0.2%); SA - salicylic acid (1 mM); Water – control.

### **Yield attributes and yield of rice**

There were significant differences in the number of grains per panicle, thousand-grain weight, straw, and grain yield, and harvest index of rice as influenced by both the exogenous application of plant growth regulating metabolites and different moisture regimes (normal and water deficit) (Table 2). Compared to normal moisture conditions, the water deficit enforced to rice plants significantly reduced the yield attributes and yield of rice under field conditions. Within the exogenous spray of small molecules, the CGCE had a significant increase in the yield attributes of grains per panicle, 1000-grain weight, grain yield and harvest index at both normal and water-deficit conditions. SCCE found the second best to improve the yield attributes and yield of rice followed by SA and the least response was noticed in the control treatment. In general, spraying of a cell extract of *Corynebacteria* followed by yeast cell extract and salicylic acid is the trend being followed in the yield attributes and yield of rice under normal and water-deficit conditions.

All the observed variables (growth attributes, drought-responsive traits, yield attributes, and yield) of rice as influenced by exogenous spraying of plant-regulating chemicals and moisture regimes were assessed for relating the variables and treatments by principal component analysis. The observation plot showing the orthogonal positions of treatments (spray chemicals and moisture regimes) (Fig. 3A) and assessed rice variables (Fig. 3B) explained by the first two components (PC1 and PC2) are presented as Fig. 4. The PC1 added 87.47% variability with PC2 added additional variability of 6.11% to a total cumulative variability of 93.57%. Two different moisture conditions imposed on rice plants were discriminated against by PCA (Fig. 3A). The water-deficit rice plants except for CGCE (WD) were orthogonally positioned and clustered together in the negative quadrant of the PCA, while SA, SCCE of normal moisture positioned in the positive quadrant and CGCE (WD) and CGCE (N) were positioned in positive for PC1 and negative for PC2 quadrant. The water (N) was positioned in the PC1 -ve PC2 +ve quadrant. All the observed growth and yield variables were positioned in PC1 +ve quadrant and positively correlated to SCCE (N), CGCE (N), SA (N), CGCW (WD) treatments. The drought-responsive variables (canopy temperature, leaf proline and catalase activities) were positioned negative to these treatments and positively related to the treatments viz., water (WD), SCCE (WD) and SA (WD). Concerning the variability contribution, all the observed variables have a significant contribution (factor loading > 0.80) (Table 3) and showed more or less equal contribution to PC1 (5 to 7% each to the total variability of the PC1).

We also noticed that the drought stress-responsive traits of rice viz., proline and antioxidant enzymes were reduced due to a combination of PGPR (ZSB15) and plant growth regulators (SA, CGCE, SCCE). Subsequently, the growth attributes such as chlorophyll and protein content along with yield attributes and yield of rice were also enhanced in rice under water deficit conditions. The present results are in agreement with several findings that the PGPR and hormonal small molecules have a synergistic effect on growth and drought mitigation of crops like wheat, sunflower, chickpea and sunflower (Khan *et al.*, 2017; Khan *et al.*, 2018a; Khan *et al.*, 2018b; Hafez *et al.*, 2019; Khan and Bano, 2019; Khan *et al.*, 2019).

### **CONCLUSION**

It is concluded that the combined effect of plant-growth regulating small molecules of microbial origin (cell extracts of yeast or *Corynebacteria*) or salicylic acid and PGPR significantly overcome the drought-induced damages of the rice. The exogenous spray of cell extracts of yeast or *Corynebacteria* or salicylic acid with soil inoculated PGPR significantly reduced the drought-responsive physiological changes i.e., catalase, proline and canopy temperature. The PGPR with plant-growth regulating small molecule showed an increase in all growth parameters of rice. This combination also increased the relative water content of the leaves. The cumulative growth and drought mitigation due to PGPR and small molecule chemicals ensured the mitigation of yield loss due to moisture stress in rice. Hence, the Integrative use of PGPR strains with exogenous plant growth regulators seems to be a promising and eco-friendly strategy for increasing drought tolerance in plants.

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