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MOLECULAR EVIDENCE OF PHYTOHORMONAL MODULATORY EFFECT OF *Serratia liquefaciens* ON RICE

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ABSTRACT

The potential plant growth promoting rhizobacterium *Serratia liquefaciens* isolate KM2 (*SL*-KM2) was evaluated for its effect on the growth and hormonal homeostatic in rice cv. 'Dandi'. The rice seeds were treated with *SL*-KM2 and sown in po;ybags. The phytohormonal analysis of rice seedlings was performed 20 days after sowing (DAS) by using UFLC techniques. The study showed that *SL*-KM2 seed treatment balanced phytohormones homeostatic wherein gibberellic acid (GA_3) and indole acetic acid (IAA) contents in leaves and root tissues were affected positively whereas abscisic acid (ABA) and jasmonic acid (JA) were negatively affected. The balanced phytohormone contents resulted in improvement in physiological parameters *viz.*, root length, root volume, shoot length and fresh weight as compared to the untreated control. The expression analysis of genes involved in phytohormones perception and signaling was performed by qRT-PCR technique at 20 DAS which further confirmed seed treatment-mediated hormonal modulation. Under seed treatment condition, the genes *GAI1, EIL1, PR1, PAL, EDS1* and *AOS2* were up-regulated in leaf tissue whereas *ERF1* gene was down-regulated. In case of root tissue, genes *GAI1, EIL1, ERF1, PAL* and *EDS1* were up-regulated whereas genes *PR1* and *AOS2* were down-regulated. Besides improving the seedling physiology, the up-regulation of *PAL* and *PR1* by *SL*-KM2 seed treatment imparted better stress tolerance in plant. This study confirms the association of phytohormone-related gene expression with observed hormonal state contributing toward enhanced physiological response in rice.

Keywords: Gene expression analysis, PGPR traits, phytohormones, rice, *Serratia liquefaciens*

INTRODUCTION

The rhizosphere is a narrow zone of soil which is specifically influenced by root system (Dobbelaere *et al*., 2003). This zone is rich in nutrients than bulk soil due to the accumulation of a variety of plant exudates such as amino acids and sugars which serve as a rich source of energy and nutrients for bacteria leading to higher bacterial population in this zone (Gray and Smith, 2005). The rhizosphere microorganisms have become important tool in guarding plant health in eco-friendly manner (Kloepper *et al.*, 1980). These plant growth-promoting rhizobacteria (PGPR) positively affect the

plant growth by direct and indirect mechanisms (Ahemad and Khan, 2009). PGPRs reportedly enhance plant growth through a variety of mechanisms like fixation of atmospheric nitrogen, production of iron-chelating siderophores, solubilization of minerals like phosphorus, and synthesis of phytohormones (Zakry *et al.,* 2012).

The ability of rhizospheric bacteria to influence plant hormonal status is likely involved in most known mechanisms of growth promotion action by PGPR (Kudoyarova *et al.*, 2019). Molecular approaches using microbial and plant mutants (with altered ability to synthesize or respond to specific phytohormones) have helped in understanding the role of phytohormone synthesis as a direct mechanism of plant growth enhancement by PGPRs (Egamberdieva *et al.*, 2017; Hassan and Bano, 2019). The growth-stimulating hormones are produced by plants under the influence of micro-organisms (Shi *et al*., 2017); whereas the metabolizing growth-inhibitory hormone leads to the plant growth promoting activity (Glick, 2014). The pivotal changes in plant expression of hormone-mediated genes have highlighted the importance of hormones in plant-microbe interactions (Ambreetha *et al.*, 2018; Jatan *et al.*, 2018). PGPRs synthesizing auxins and cytokinins that interfere plant ethylene synthesis have been identified. Only 1 to 2% of bacteria promote plant growth in rhizosphere (Antoun and Kloepper, 2001). PGPR can alter root architecture and promote plant development by producing different phytohormones like IAA, gibberellic acid, cytokinins, etc. (Kloepper *et al*. 2007, Sun *et al.*, 2018). The PGPR modulate phytohormone level in plant by regulating the expression of various genes *viz*., gibberellic acid insensitive (*GAI*), ethylene insensitive 3-like EI (*EIL1*), ethylene-responsive factor (*ERF*), pathogenesis-related gene (*PRG*), phenylalanine ammonia-lyase (*PAL*), enhanced disease susceptibility 1-like (*EDS1*) and allene oxide synthase (*AOS*) show influence both at biochemical and morphological levels. The present study was aimed to assess the influence of transcript level change in reported regulatory genes with phytohormones level and its effect on growth in model plant system rice.

MATERIALS AND METHODS

Plant material

The seeds of rice (*Oryza sativa*) cv. 'Dandi' was procured from Agronomy Farm, N.M. College of Agriculture, Navsari Agricultural University (NAU), Navsari, Gujarat (India). The seeds were surface sterilized with 0.01% $HgCl_2$ for 1 min followed by four rinsing with autoclaved double distilled water to remove all the traces of $HgCl_2$.

PGPR treatment

The potential PGPR isolate (*Serratia liquefaciens* isolate KM2) was procured from the Department of Plant Pathology, N.M. College of Agriculture, NAU, Navsari and used for seed treatment of surface sterilized rice seeds cv. 'Dandi'. The overnight grown culture (1×10^8 cfu mL^{-1}) was used for seed treatment @ 10 mL kg^{-1}. The treated seeds were shade-dried and then sown in polybags containing aseptic potting mixture (50:50, sand:soil). Ten seeds were sown per polybag. The experiment was conducted in a complete randomized design and each treatment was replicated 10 times. The bags were irrigated and nourished with Hoagland's solution as and when required. A suitable untreated control was also maintained.

Physiological analysis

The root length, root volume, shoot length and fresh weight were measured 10 and 20 days after sowing (DAS). The uprooted plants were washed under clean water and then air-dried before analysis. The root and shoot length were measured with a scale from below and above the collar region at root/shoot junction. The root volume was measured by dipping the roots into measuring cylinder and calculating the rise in volume. The fresh weight was measured using weighing balance.

Molecular and phytohormonal analysis

For molecular and phytohormonal analysis, the leaves and root tissue were collected at 20 DAS. The phytohormones *viz.,* gibberellic acid (GA_3), indole acetic acid (IAA), abscisic acid (ABA) and jasmonic acid (JA) were analyzed as per the method of Yuan *et al.* (2010) using Ultra-Fast Liquid Chromatography (UFLC; Shimadzu, Japan) equipped with PDA detector 2800 and C-18 column. The samples were separated using methanol: water (60:40) mobile phase having a flow rate of 1.0 mL min^{-1}, followed by visualization with UV visible detectors (GA, IAA and JA at 208 nm and ABA at 265 nm). The detection and quantification of phytohormones were done by comparison with standards based on retention time and area-concentration response, respectively.

The RNA was extracted by *TRIzol* method following the instruction of manufacturer (Invitrogen). The QuantiTect reverse transcription kit (QIAGEN) was used for cDNA synthesis with integrated removal of genomic DNA contamination as per the manufacturer's instructions. The expression analysis of PGPR trait-related genes *viz.*, gibberelic acid insensitive (*GAI*): F- GGATCC AAATCCCAACCTATCC, R- GTACTCGCGCTTCATGATCTC, ethylene insensitive 3-like E1 (*EIL1)*: F- ATCACCAGCGCCATATCGTT, R- CACGGTTGTTCAGCATCAGC, ethylene-responsive factor 1 *(ERF1)*: F- CATATCACCTTGACGCCCCA, R- ACCCTCACAAACTCACTCGG, pathogenesis-related gene 1 *(PR1)*: F- TCGTATGCTATGCTACGTGTTT, R- CAC TAAGCAAAT ACGGCTGACA, phenylalanine ammonia lyase (*PAL)*: F- GCGGATTCAATGAGAGGTAGTC, R-CCGCAGCCACTTGAGATATT, enhanced disease susceptibility 1-like (*EDS1)*: F- CATTCCAAG AACGAGGACACTG, R-CAAGACTCAAGGCTAGAACCGA and allene oxide synthase 2 (*AOS2)*: F- CTCGTCGGAAGGCTGTTGCT, R- ACGATTGACGGCGGA GGTT were done using Quanti-Fast™ SYBR® Green PCR kit (QIAGEN) through ABI 7300 real time cycler (ABI, USA). The relative quantification of genes was calculated as fold changes in gene expression using actin (*ACT;* F-CCCTGCTATGTATGTCGCTATT, R- GAAGGGCATAACCCT CGTAAA) as an endogenous control for normalization of gene expression data analyzed by Applied Biosystems 7300 Real-Time PCR System software SDS v1.4.

The standard basic descriptive statistical analysis was performed for physiological and phytohormones study as per Panse and Sukhatme (1978).

RESULTS AND DISCUSSION

The *SL*-KM2 seed treatments showed positive influence on root, shoot and overall plant growth at 10 and 20 DAS (Fig. 1). The maximum root length (23.67 cm), shoot length (16.58 cm), root volume (1.356 mL) and fresh weight (0.42 g) was noticed in *SL*-KM2 seed treatments at 10 DAS. The same trend was observed at 20 DAS also. The phytohormones analysis carried out at 20 DAS showed that GA_3 and IAA were positively influenced by seed treatments whereas ABA and JA in leaf and root tissues were negatively influenced (Fig. 3). The GA_3 content was higher in leaf tissue as compared to the root tissue, whereas IAA content was higher in root tissue as compared to the leaf tissue. The highest GA_3 content of 56.12 nM g^{-1} FW and IAA content of 27.53 nM g^{-1} FW were found in *SL*-KM2 seed treatment in leaf and root tissue, respectively. The highest ABA content of 3.01 nM g^{-1} FW and JA content of 7.85 nM g^{-1} FW were noticed in leaf tissue of untreated rice plant. The *SL*-KM2 seed treatment decreased ABA and JA contents in both leaf and root tissues. Further, the contents of these phytohormones were higher in leaf tissue as compared to root tissue. The balanced phytohormone content favoured the vigorous seedling growth.

The amplification plot and dissociation analysis of *GAI, EIL, PR1, PAL, EDS1, AOS2* and *ERF1* genes showed single melt curve which indicated that only specific product was amplified during qPCR analysis (data not shown). The *SL*-KM2 seed treatment influenced the expression of different PGPR trait-related genes in both root and leaf tissue of rice at 20 DAS (Fig. 4). The *GAI* gene

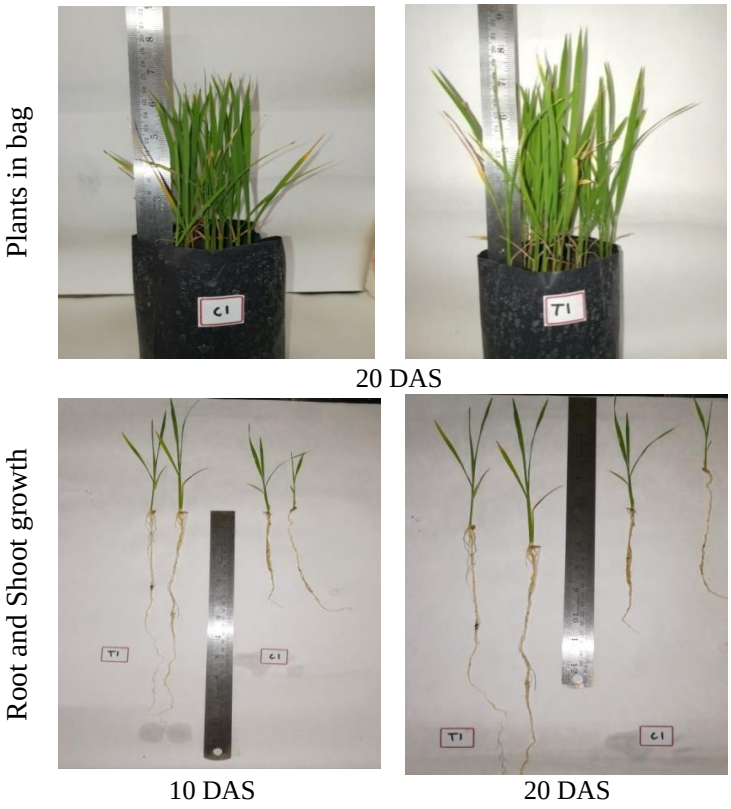


Fig. 1: Effect of *SL*-KM2 seed treatment on rice growth; Note: C_1: So - without *SL*-KM2 seed treatment: T_1: Si - with *SL*-KM2 seed treatment

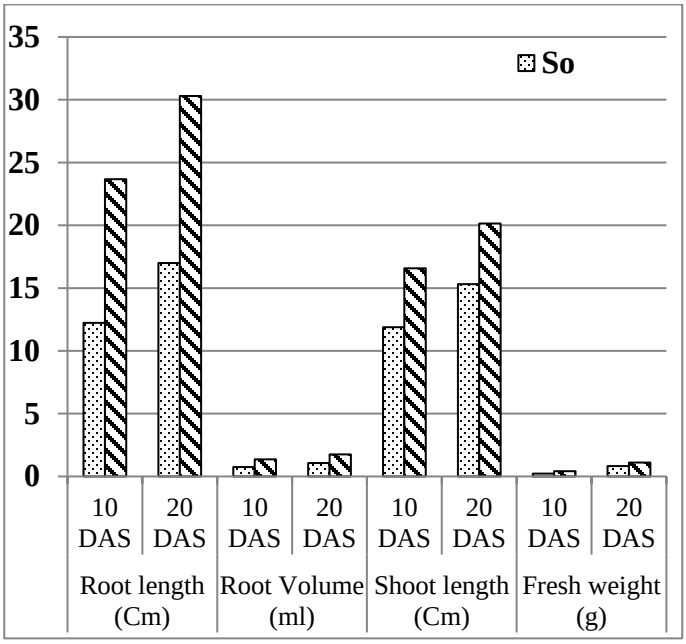
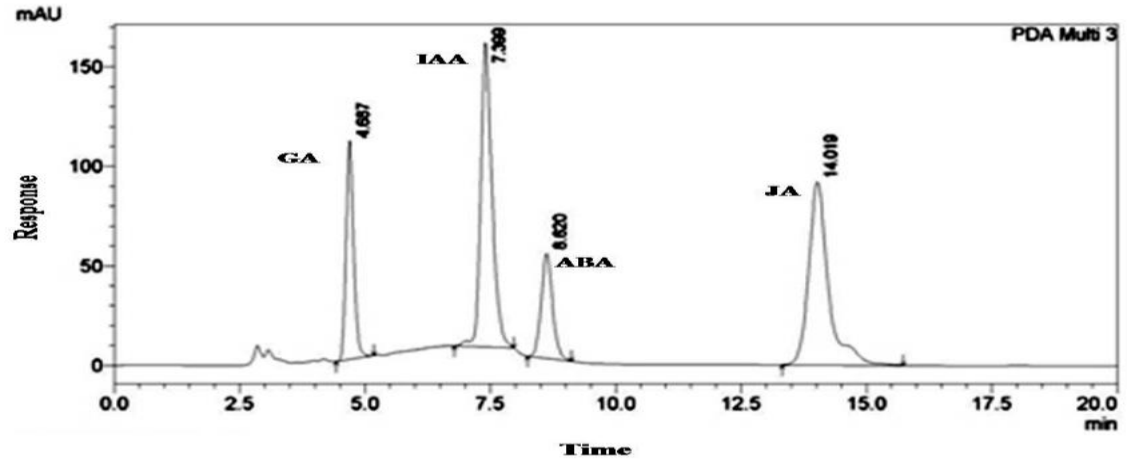


Fig. 2: Effect of *SL*-KM2 seed treatment on physiological parameters of rice at different time interval; So - without SL-KM2 seed treatment; Si - with *SL*-KM2 seed treatment

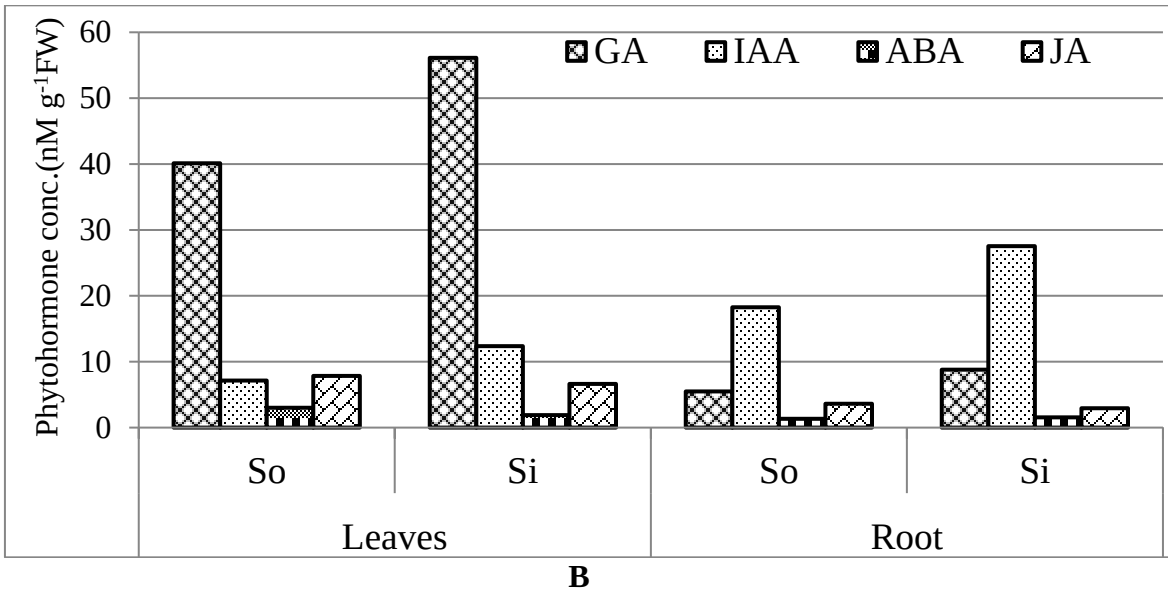
was up-regulated in leaves (0.04 Rq) and root tissue (0.76 Rq). There was significant up-regulation of *EIL1* gene in leaves tissue (3.46 Rq) and it was also up-regulated in root tissue (0.25 Rq) by seed treatment. The *ERF1* gene was down-regulated in leaves tissue (-4.73 Rq) whereas up-regulated in root tissue (0.25 Rq). The *PR1* gene expression was 1.55-fold higher in leaf tissue whereas it was down-regulated in root tissue (-1.88 Rq) by seed treatment. The *SL*-KM2 seed treatment increased *PAL* gene expression in leaf (2.48 Rq) and root tissue (0.54 Rq). The *EDS1* gene was also up-regulated in leaf tissue (0.72 Rq) and root tissue (0.31 Rq). There was up-regulation observed for *AOS2* gene in leaves (1.92 Rq) whereas it was down-regulated in root tissue (-1.01 Rq).

The interaction between microbes and the host plant is a mutualistic interaction that improves plant growth under stress through the induction of osmoregulation, hormonal balance, biochemical processes and changes in metabolic interfaces among partners (Nadeem *et al.*, 2014; Park *et al.*, 2017, Kudoyarova *et al.*, 2019). In this study, *SL*-KM2 seed treatments improved the seedling physiology which coincided with the hormonal balanced in rice. Reports reveal that some bacteria in various crops may produce several types of phytohormones in plant tissues that interact to modulate important physiological processes in plants, including hormonal balance (Asaf *et al.*, 2017, Zhao and Zhang, 2015, Cohen *et al*., 2015, Ambreetha *et al.*, 2018; Jatan *et al.*, 2018).

In present study, *GAI1*, *EIL1*, *PR1*, *PAL*, *EDS1* and *AOS2* genes were up-regulated in leaf tissue whereas *ERF1* was down-regulated under seed treatment condition. In case of root tissue, *GAI1*, *EIL1*,



A



B

Fig. 3: A) Chromatogram of standard phytohormones analyzed through UFLC; B) Effect of *SL*-KM2 seed treatment on phytohormones at 20 DAS; So - without *SL*-KM2 seed treatment; Si - with *SL*-KM2 seed treatment

ERF1, *PAL* and *EDS1* were up-regulated whereas *PR1* and *AOS2* were down-regulated. The *OsEIL1* is probable transcription factor acting as a positive regulator in the ethylene response pathway. It is required for ethylene responsiveness in adult plant tissues and binds a primary ethylene response element present in the ethylene-response-factor1 (*ERF1*) promoter with consequence to activate the transcription of this gene (Chao *et al*., 1997; Solano *et al*., 1998). The least expression of *OsEIL1* found in root tissue reports lower cellular ethylene production in root tissue that further supported by lower expression of ethylene-response-factor1 *(ERF1).* In leaf tissue the manifold increase in *EIL1* did not lead to enhanced expression of *ERF1* and reveals lower cellular ethylene in leaves tissue which is further evidenced from shoot morphology. Mao *et al*. (2006) in transgenic rice plants found that the over-expression of *OsEIL1* exhibited short root, coiled primary root and slightly short shoot phenotype as well as elevated response to exogenous ethylene and acted as a positive regulator of ethylene response. Inoculation of wheat plants with auxin-producing bacteria *Paenibacillus illinoisensis* IB 1087 and *Pseudomonas extremaustralis* IB-K13-1A enhanced root mass and root auxin concentrations (Kudoyarova *et al.*, 2017). Mei *et al*. (2006) found that the transgenic rice lines carrying the *OsAOS2* transgene exhibited enhanced activation of pathogenesis-related (PR) genes

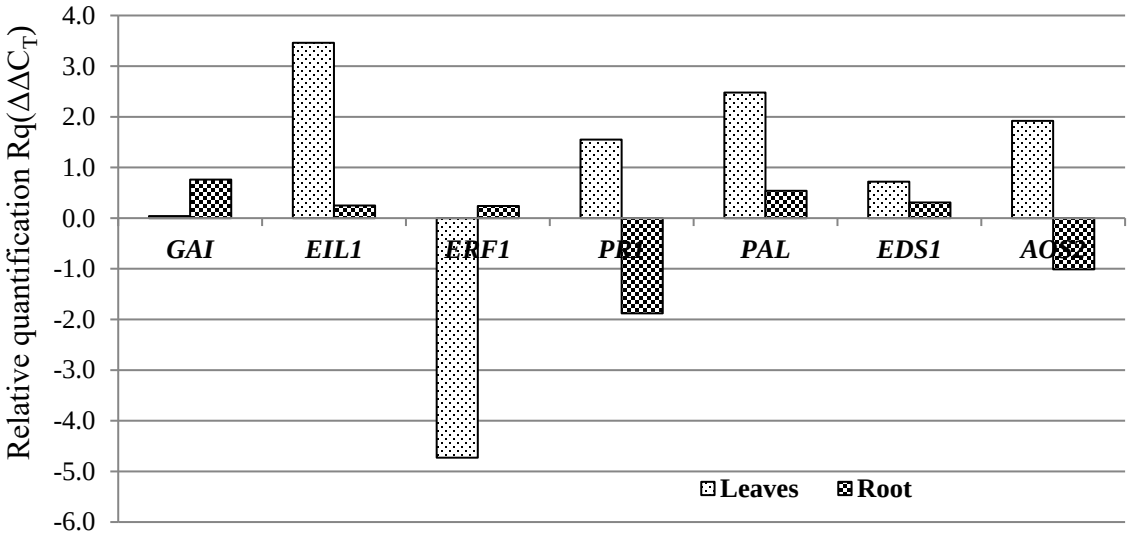


Fig. 4: Effect of *SL*-KM2 on expression of PGPR traits related genes in rice at 20 DAS

such as *PR1a*, *PR3*, and *PR5* and increased resistance to *M. grisea* infection. In present study, the *AOS2* gene was down-regulated in root tissue by seed treatment that influenced the *PR1* gene expression and it was also down regulated. In case of leaf tissue, the up-regulation of *AOS2* gene was observed that further improved the *PR1* gene expression. The seed treatment of rice with *SL*-KM2 lead to the up-regulation of *PAL* gene in leaf and root tissues. Tanaka *et al.*, (2003) also reported increase in *PAL* enzymatic activities by inoculation of rice with an incompatible strain of *Acidovorax avenae*. The slight increase in *GAI1* expression was observed in root and leaves tissue; however, its phenotypic effect on plant was insignificant. Fu *et al*. (2001) reported that high-level expression of *GAI* caused dwarfism and reduced GA responses. Falk *et al*. (1999) suggested a possible role of SA and *EDS1* in potentiating the defense response. This gene was up-regulated in leaf and root tissues by seed treatment. Overall, the PGPR bacteria stimulate plant growth by influencing phytohormonal status.

The seed treatment with *Serratia liquefaciens* isolate KM2 in rice improved the seedling vigour by properly balancing the hormonal homeostasis in plant. The gene expression analysis also supported the morphological and biochemical data. This study reported the expression regulation of PGPR traits related genes in response to seed treatments with micro-organism contributing toward hormonal homeostasis and plant growth.

REFERENCES

Ahemad, M. and Khan, M.S. 2009. Effect of insecticide-tolerant and plant growth-promoting *Mesorhizobium* on the performance of chickpea grown in insecticide stressed alluvial soils. *Journal of Crop Science and Biotechnology*, **12**(4): 217-226.

Ambreetha, S., Chinnadurai, C., Marimuthu, P. and Balachandar, D. 2018. Plant-associated *Bacillus* modulates the expression of auxin-responsive genes of rice and modifies the root architecture. *Rhizosphere*, **5**: 57-66.

Antoun, H. and Kloepper, J.W. 2001. Plant growth promoting rhizobacteria. pp. 1477-1480. **In**: *Encyclopedia of Genetics* (eds. S. Brenner and J.F. Miller). Academic Press, New York, USA.

Asaf, S., Khan, M.A., Khan, A.L., Waqas, M., Shahzad, R. and Kim, A.Y. 2017. Bacterial endophytes from arid land plants regulate endogenous hormone content and promote growth in crop plants:

an example of *Sphingomonas* sp. and *Serratia marcescens*. *Journal of Plant Interactions,* **12**: 31-38.

Chao, Q., Rothenberg, M., Solano, R., Roman, G., Terzaghi, W. and Ecker, J.R. 1997. Activation of the ethylene gas response pathway in *Arabidopsis* by the nuclear protein ethylene-insensitive 3 and related proteins. *Cell*, **89**: 1133-1144.

Cohen, A.C., Bottini, R., Pontin, M., Berli, F.J., Moreno, D. and Boccanlandro, H. 2015. *Azospirillum brasilense* ameliorates the response of *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. *Physiologia Plantarum.* **153**: 79-90.

Dobbelaere, S., Vanderleyden, J. and Okon, Y. 2003. Plant growth-promoting effects of diazotrophs in the rhizosphere. *Critical Reviews in Plant Sciences,* **22**: 107-149.

Egamberdieva, D., Wirth, S.J., Alqarawi, A.A., Abd_Allah, E.F. and Hashem, A. 2017. Phytohormones and beneficial microbes: Essential components for plants to balance stress and fitness. *Frontiers in Microbiology,* **8**: 2104

Falk, A., Bart J.F., Louise, N.F., Jonathan D.G.J., Michael J.D. and Jane E.P. 1999. EDS1, an essential component of R gene-mediated disease resistance in *Arabidopsis* has homology to eukaryotic lipases. *Proceedings of the National Academy of Sciences,* **96**: 3292-3297.

Fu, X., Sudhakar, D., Peng, J., Richards, D.E., Christou, P. and Harberd, N.P. 2001. Expression of *Arabidopsis GAI* in transgenic rice represses multiple gibberellin responses. *Plant Cell*, **13**: 1791-1802.

Glick, B.R. 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiological Research,* **169**: 30-39.

Gray, E.J. and Smith, D.L. 2005. Intracellular and extracellular PGPR: Commonalities and distinctions in the plant-bacterium signaling processes. *Soil Biology and Biochemistry,* **37**: 395-412.

Hassan, T.U. and Bano, A. 2019. Construction of IAA-deficient mutants of *Pseudomonas moraviensis* and their comparative effects with wild type strains as bio-inoculant on wheat in saline sodic soil. *Geomicrobiology Journal,* **36**: 376-384.

Jatan, R., Chauhan, P.S. and Lata, C. 2018. *Pseudomonas putida* modulates the expression of miRNAs and their target genes in response to drought and salt stresses in chickpea (*Cicer arietinum* L.). *Genomics*. **111**: 509-519.

Kloepper, J.W. 1993. Plant growth-promoting rhizobacteria as biological control agents. pp 255–274. In: Metting FB Jr (ed) Soil microbial ecology. Dekker, New York,

Kloepper, J.W., Gutierrez-Estrada, A. and McInroy, J.A. 2007. Photoperiod regulates elicitation of growth promotion but not induced resistance by plant growth-promoting rhizobacteria. *Canadian Journal of Microbiology*, **53**: 159-167.

Kloepper, J.W., Leong, J., Teintze, M. and Schroth, M.N. 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*, **286**(5776): 885-886.

Kudoyarova, G.R., Vysotskaya, L.B., Arkhipova, T.N., Yu, K.L., Galimsyanova, N.F. and Sidorova, L.V. 2017. Effect of auxin producing and phosphate solubilizing bacteria on mobility of soil phosphorus, growth rate, and P acquisition by wheat plants. *Acta Physiologia Plantarum,* **39**: 253-265.

Kudoyarova, G., Arkhipova, T.N., Korshunova, T., Bakaeva, M., Loginov, O. and Dodd, I.C. 2019. Phytohormone mediation of interactions between plants and non-symbiotic growth promoting bacteria under edaphic stresses. *Frontiers in Plant Science*, **10**: 1368-1379.

Mao, C., Wang, S., Jia, Q. and Wu, P. 2006. *OsEIL1*, a rice homolog of the *Arabidopsis EIN3* regulates the ethylene response as a positive component. *Plant Molecular Biology*, **61**: 141-152.

Mei, C., Min, Q., Guangyao, S. and Yinong, Y. 2006. Inducible over expression of a rice *allene oxide synthase* gene increases the endogenous jasmonic acid level, PR gene expression, and host resistance to fungal infection. *Molecular Plant-Microbe Interactions,* **19**: 1127-1137.

Nadeem, S.M., Ahmad, M., Zahir, Z.A., Javaid, A. and Ashraf, M. 2014. The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnology Advances,* **32**: 429-448.

Panse V.G. and Sukhatme P.V. 1978. *Statistical Methods for Agriculture Workers.* Indian Council of Agricultural Research, New Delhi, India.

Park, Y.G., Mun, B.G., Kang, S.M., Hussain, A., Shahzad, R. and Seo, C.W. 2017. *Bacillus aryabhattai* SRB02 tolerates oxidative and nitrosative stress and promotes the growth of soybean by modulating the production of phytohormones. *PLOS ONE,* 12: e0173203.

Shi, T.Q., Peng, H., Zeng, S.Y., Ji, R.Y., Shi, K. and Huang, H. 2017. Microbial production of plant hormones: opportunities and challenges. *Bioengineered*, **8**(2) :124-128.

Solano, R., Stepanova, A., Chao, Q. and Ecker, J.R. 1998. Nuclear events in ethylene signaling: A transcriptional cascade mediated by ethylene-insensitive 3 and ethylene-response-factor 1. *Genes and Development*, **12**(23): 3703-3714.

Sun, S.L., Yang, W.L., Fang, W.W., Zhao, Y.X., Guo, L. and Dai, Y.J. 2018. The plant growth-promoting rhizobacterium *Variovorax boronicumulans* CGMCC 4969 regulates the level of indole-3-acetic acid synthesized from indole-3-acetonitrile. *Applied and Environmental Microbiology*, **84**(16): e00298-18.

Tanaka, N., Che, F.S., Watanabe, N., Fujiwara, S., Takayama, S. and Isogai, A. 2003. Flagellin from an incompatible strain of *Acidovorax avenae* mediates H_2O_2 generation accompanying hypersensitive cell death and expression of *PAL*, *Cht*-1, and *PBZ1*, but not of *Lox* in rice. *Molecular Plant-Microbe. Interactions,* **16**: 422-428.

Yuan, H., Zhao, K., Lei, H., Shen, X., Liu, Y., Liao, X. and Li, T. 2010. Genome-wide analysis of the GH3 family in apple (*Malus* × *domestica*). *BMC Genomics*, **14**: 1-8.

Zakry, F.A.A., Shamsuddin, Z.H., Rahim, K.A., Zakaria, Z.Z. and Rahim, A.A. 2012. Inoculation of *Bacillus sphaericus* UPMB-10 to young oil palm and measurement of its uptake of fixed nitrogen using the ^{15}N isotope dilution technique. *Microbes and Environment,* **27**: 257-262.

Zhao, L. and Zhang, Y. 2015. Effects of phosphate solubilization and phytohormone production of *Trichoderma asperellum* Q1 on promoting cucumber growth under salt stress. *Journal of Integrative Agriculture,* **14**: 1588-1597.