Plant-rhizobacteria interactions mitigates drought stress

Dr. Sk. Z. Ali
Assistant Professor- Microbiology,
Agri Biotech Foundation,
PJTSAU Campus, Rajendranagar,
Hyderabad – 500030, Telangana
State, India

Email: skzali28@gmail.com
www.abfindia.org

4th Asian PGPR Conference
May 3 – 6, 2015
Hanoi, Vietnam
Most difficult task ahead - to feed the world’s ever growing population

Demand for food – expected to rise by 3-5 time

Current food production has to increase by 50% by 2030

This task is becoming difficult due to the immediate threat of climate change

Global warming and its associated effects are imposing abiotic stresses
Global projections suggest that the temperatures may rise by 0.6 to 2.5°C by 2050 and 1.4 to 5.8°C by 2100.

Climate Change Induced Abiotic Stresses in India

- Water: Deficit (Drought)/ Excess (Flooding)
- Temperature: High/Low (chilling and Freezing)
- Chemical: Salts, Ions, Gases and Herbicides.
- Radiations: IR, Visible, UV, Ionizing (X-rays and Y-rays).

Effects
Agricultural Productivity
One of the major constraints that limit crop production and quality
54% of India Faces High to Extremely High Water Stress

www.indiawatertool.in
Water, comprising 80-90% of the biomass of non-woody plants

Central molecule in all physiological processes of plants - transporting metabolites and nutrients

Drought is a situation that lowers plant water potential and turgor to the extent that plants face difficulties in executing normal physiological functions
Subjected water deficit plants go through a cascade of metabolic alterations.
Coping Strategies

Microbes play a significant role in enhancing adaptation of plants to abiotic stresses

Highly relevant for us as a low cost input as compared to cost intensive management practices
What Microbes?

Bacterial endophytes contribute to abiotic stress adaptation in pepper plants (*Capsicum annuum* L.)

A.H. Sziderics, F. Rasche, F. Trognitz, A. Sessitsch, and E. Wilhelm

The Plant-Growth-Promoting Rhizobacterium *Paenibacillus polymyxa* Induces Changes in *Arabidopsis thaliana* Gene Expression: A Possible Connection Between Biotic and Abiotic Stress Responses

Salme Timmusk and E. Gerhart H. Wagner

Virus infection improves drought tolerance

Ping Xu, Fang Chen, Jonathan P. Mannas, Tracy Feldman, Lloyd W. Sumner and Marilyn J. Roossinck
The S. R. Noble Foundation, Ardmore, OK 73401, USA

Seed Treatment with *Trichoderma harzianum* Alleviates Biotic, Abiotic, and Physiological Stresses in Germinating Seeds and Seedlings

Fatemeh Mastouri, Thomas Björkman, Gary E. Harman

Functional Biotechnology

Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: Effectiveness of autochthonous or allochthonous strains

N. Ortiz², E. Armada², E. Duque², A. Roldán², R. Azcón²,³
Where they are?

Rhizosphere

Rhizosphere microbiota - key component of plant fitness

In the 1g soil around plant root; more than $10^9$ bacteria and many others
Plant-microbe interactions

Root colonization by PGPR

- Compete with indigenous microorganisms
- Environmental factors – biotic and abiotic

Bacterial traits contribute to root colonization:

- chemotaxis to seed and root exudates
- production of pili or fimbriae
- production of specific cell surface components
- ability to use specific components of root exudates,
- protein secretion
- biofilm-forming ability of the microbes
- quorum sensing
Root exudates attract *Rhizobium* and flavonoids prime the microsymbionts for the interaction. Host plants initially recognize rhizobia as potential invaders; pattern-recognition receptors (PRR) in the host recognize microbe-associated molecular patterns (MAMPs (flagellin) yellow-colored shapes) and a signaling cascade is initiated that results in MAMP-triggered immunity (MTI). Lipopolysaccharide polysaccharides (LPS) function early during the interaction, most likely as extracellular effectors to facilitate immune avoidance/bypassing. At later stages, the establishment of the symbiotic program in the plant cells, which is activated upon perception of the rhizobial nodulation (Nod) factors, counteracts the MTI. Rhizobial effectors that are secreted through the type III secretion system (brown-colored shapes) may assist in the suppression of the MTI response or act as symbiotic determinants.

Zamioudis and Pieterse (2012), MPMI
Non-symbiotic beneficial interactions

Root exudates attract PGPR for the interaction.

Host plants initially recognize PGPR as potential invaders; pattern-recognition receptors (PRR) in the host recognize microbe-associated molecular patterns (MAMPs (flagellin) yellow-colored shapes) and a signaling cascade is initiated that results in MAMP-triggered immunity (MTI). Lipo polysaccharides (LPS) function early during the interaction, most likely as extracellular effectors to facilitate immune avoidance/bypassing. Host plant exhibit ethylene (ET) induce defence immune response. As some of the *Pseudomonas* have the ability to produce ACC deaminase, lowers the concentration of ACC the precursor for ET there-by reduce the ET concentration in plants that lowers ET dependent defense response.

Attenuation of SA signaling via hormonal cross-talk mechanisms.

Zamioudis and Pieterse (2012), MPMI
Mechanisms behind plant drought tolerance enhancement by microbes

1-aminocyclopropane-1-carboxylate deaminase (ACCd)

Ethylene production is induced during certain stages of plant growth such as germination, ripening of fruits, abscission of leaves and senescence of flowers.

Ethylene production can also be induced by a variety of external aspects such as mechanical wounding, environmental stresses, and certain chemicals.
Bacterial volatiles promote growth promotion in *Arabidopsis*

Quantification of growth promotion in *A. thaliana* with exposure to bacterial volatiles

Bacterial volatiles promote Induced Systemic Tolerance (IST) to drought in Arabidopsis

Bacterial volatile 2R,3R-butanediol was a major determinant in inducing resistance to drought in Arabidopsis by stomatal closure

Cho et al. (2008) MPMI
What plant signals involved in PGPR mediated IST?

Major player is Salicylic acid signaling pathway.

Cho et al. (2008) MPMI
PGPR mediated root phospholipids alterations exposed to drought stress

Water deficit increased phosphatidylcholine (PC) content and diminished that of phosphatidylethanolamine (PE) in uninoculated seedlings. *Azospirillum* inoculation showed no significant change.

*Azospirillum* inoculation protected wheat seedlings from drought stress through changes in the fatty acid distribution profiles of PC and PE major root phospholipids.

PGPR treatment enhance the mesophyll tissue in wheat plants under drought stress

Treating wheat plants with *Azotobacter chrocoocum* and *Pseudomonas fluorescens* significantly increased thickness of epidermis, ground, mesophyll and phloem tissues.

Transverse sections of wheat (stained with safranin and light green) treated with bacteria. A. control, B. *A.chrocoocum*, C. *P.fluorescens*

El-Afry et al. (2012), Acta Biologica Szegediensis
Modification of plant root architecture by PGPR

*Pseudomonas putida* produces significant amounts of nitric oxide, which has been shown to act as a signaling molecule in an IAA induced pathway involved in lateral adventitious root development resulting in increased root surface area and water use efficiency.

Molina-Favero et al. (2008) MPMI
PGPR induces changes in plant gene expression

- Inoculation with *Paenibacillus polymyxa* enhanced the drought tolerance of *Arabidopsis thaliana*.

- By using RNA display, drought-response gene, *EARLY RESPONSE TO DEHYDRATION 15 (ERD15)*, was detected inoculated plants compared to uninoculated controls.

Timmusk and Wagner (1999), MPMI
Transcriptome studies of IST Elicited by *P. chlororaphis* in *Arabidopsis* under drought stress

- Root colonization increased the expression levels of genes associated with defense, reactive oxygen species, auxin- and jasmonic acid-responsive genes, but decreased ethylene and abscisic acid signaling genes
- Jasmonic acid-marker genes, VSP1 and pdf-1.2, the salicylic acid regulated gene, PR-1, and the ethylene-response gene, HEL, were up-regulated in plants colonized by *P. chlororaphis*, but differed in their response to drought stress

Hierarchical cluster and expression pattern of genes expressed differentially.

Cho et al. (2013), Plant Pathol. J

RT-PCR analysis
PGPR activates plant antioxidant defense systems under drought stress

PGPR induced higher activity of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and lower level of hydrogen peroxide ($H_2O_2$), malondialdehyde (MDA) in rice plants under drought stress compared to un-inoculated

Gusain et al. (2015), Afr. J. Biotechnol
PGPR enhanced compatible solutes production and accumulation

Proline - up regulation of proline biosynthesis pathway, helps in maintaining cell water status, protects membranes and proteins from stress
(Sandhya et al. (2010), Plant Growth Regul; Qudsia Bano et al. (2013), Pak.J.Bot.)

Sugars – osmoprotectant (stabilize cellular membranes), substrate in biosynthesis processes, energy production, being the products of hydrolytic metabolic pathways, they may also contribute as regulatory signal molecules for metabolic regulation
(Sheen et al., 1999; Smeekens, 2000; Gibson, 2005)

Amino acids - Outcome of hydrolysis of proteins, that crop up in response to alterations of osmotic adjustment; particularly the breakdown of structural proteins into the constituent amino acids
(Iqbal et al. (2011), Plant Growth Regul)
Research work

- **Soil structure** - Soil aggregate stability - Exopolysaccharides (EPS) production by bacteria

- *Pseudomonas* spp. - catabolic versatility, root colonizing ability and produce a wide range of enzymes and metabolites that favor the plant withstand under varied abiotic stress conditions

- **Isolation of indigenous *Pseudomonas* spp. from stressed ecosystems** - may help in the selection of stress-adapted strains

- **Plant performance** - selected microbial strains on morphological, physiological and biochemical parameters of plants under drought stress conditions

- **Molecular mechanisms** – to elucidate the mechanism behind the drought tolerance by microbes using transcriptomic approaches – NGS method
Soil sample collection
Isolation
Focus on *Pseudomonas* sp.

Screening for PGPR traits
- IAA
- Gibberellic Acid
- P-solubilization
- Siderophore
- HCN

Screening for drought tolerance

Phenotypic characterization & Genotypic Characterization

16S rDNA

BLAST analysis and gene submission to GENBANK

Matric potential (MPa)

Number of isolates

Alfisol  Vertisol  Inceptisol  oxisol  Aridisol

Screening for PGPR traits

IAA

Gibberellic Acid

P-solubilization

Siderophore

HCN

ACC deaminase activity
Physiological parameters of *Pseudomonas* sp. under drought stress

<table>
<thead>
<tr>
<th>Strains</th>
<th>Exopolysaccharide (mg mg(^{-1}) protein)</th>
<th>Protein (mg g(^{-1}) DW)</th>
<th>Total free amino acids (µmol g(^{-1}) DW)</th>
<th>Proline (µmol g(^{-1}) DW)</th>
<th>Total soluble sugars (µmol g(^{-1}) DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS</td>
<td>DS</td>
<td>NS</td>
<td>DS</td>
<td>NS</td>
</tr>
<tr>
<td>BV-P13</td>
<td>2.4 ± 0.02</td>
<td>14.9 ± 0.03</td>
<td>21.67 ± 0.94</td>
<td>6.89 ± 0.55</td>
<td>11.56 ± 0.52</td>
</tr>
<tr>
<td>GRFHAP-P14</td>
<td>3.7 ± 0.41</td>
<td>20.7 ± 0.14</td>
<td>21.23 ± 0.61</td>
<td>6.47 ± 1.02</td>
<td>13.03 ± 0.14</td>
</tr>
<tr>
<td>GAP-P45</td>
<td>7.4 ± 0.32</td>
<td>40.0 ± 0.14</td>
<td>23.57 ± 1.08</td>
<td>10.95 ± 0.03</td>
<td>18.87 ± 0.82</td>
</tr>
<tr>
<td>GRFHYP52</td>
<td>4.2 ± 0.02</td>
<td>25.4 ± 0.31</td>
<td>22.56 ± 1.90</td>
<td>9.68 ± 0.15</td>
<td>16.03 ± 0.52</td>
</tr>
<tr>
<td>WAPP53</td>
<td>3.9 ± 0.09</td>
<td>21.8 ± 0.45</td>
<td>21.13 ± 1.00</td>
<td>7.34 ± 0.25</td>
<td>14.13 ± 0.50</td>
</tr>
</tbody>
</table>

Numerical values are mean ± SD of six independent values; *NS* non-stressed, *DS* drought-stressed

Sandhya et al. 2010, Arch. Microbiol
Soil aggregation inoculated with EPS producing isolates. a, bulk soil; b and c, soil aggregates 2 mm and 1 mm

Effect of inoculation of *Pseudomonas* spp. isolates on EPS content and aggregate stability at different incubation periods under (a) non-stress and (b) drought stress.
EPS in relation to aggregate stability in inoculated and uninoculated diverse soil types

Scanning electron microscopy for soil aggregates

**Control**

**GAP-P45**
HPLC chromatograms of exopolysaccharide components in *Pseudomonas* spp. isolate GAP-P45

Identification of EPS genes in drought tolerant *Pseudomonas* spp. isolate GAP-P45

*algD* gene for Alginate

Blast analysis showed 100% similarity to *P. putida* KT2440 *algD* gene (Acc.No. AE015451)
## Screening for PGP traits

<table>
<thead>
<tr>
<th>Plant growth promoting properties</th>
<th>Non-stressed conditions</th>
<th>Drought-stressed conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC deaminase</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydrogen cyanide</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Indole acetic acid</td>
<td>329.33 (±10.12) μg mg⁻¹ protein</td>
<td>171 (±2.60) μg mg⁻¹ protein</td>
</tr>
<tr>
<td>Gibberellins</td>
<td>382.7 (±2.1) μg mg⁻¹ protein</td>
<td>105.0 (±13.5) μg mg⁻¹ protein</td>
</tr>
<tr>
<td>P-solubilization</td>
<td>69.3 (±6.7) μg ml⁻¹</td>
<td>41.7 (±4.0) μg ml⁻¹</td>
</tr>
<tr>
<td>Siderophore</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Alleviation of drought stress effects in sunflower and maize seedlings by the exopolysaccharide producing PGP *Pseudomonas* spp. isolate GAP-P45

Sandhya et al. 2010, Plant Growth Regul.
Scanning electron microscopic micrographs of sunflower roots colonized by *P. putida* strain GAP-P45

Root adhering soil in sunflower seedlings

a, uninoculated non-stress; b, inoculated non-stress; c, uninoculated drought-stress; d, inoculated drought-stress

Fig. 1 Growth promotion of sunflower seedlings inoculated with *P. putida* strain GAP-P45 (a) root and shoot length; (b) root and shoot dry biomass. *NSUI* non-stressed uninoculated, *NSI* non-stressed inoculated, *DSUI* drought-stressed uninoculated, *DSI* drought-stressed inoculated. Values with different letters are significantly different at $P<0.05$ in all the treatments.
Effect of *Pseudomonas* sp. strain GAP-P45 inoculation on soil structure and physiology of sunflower seedlings

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root adhering soil dry weight per root tissue ratio (mg/mg)</th>
<th>Exopolysaccharide (mg/plant)</th>
<th>Aggregate stability (%)</th>
<th>Mean weight diameter (mm)</th>
<th>Relative water content (%)</th>
<th>Soil moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-stressed uninoculated</td>
<td>18.23a (±0.49)</td>
<td>14.37a (±0.51)</td>
<td>30.00a (±2.00)</td>
<td>0.170a (±0.02)</td>
<td>53.70a (±1.60)</td>
<td>12.3a (±0.04)</td>
</tr>
<tr>
<td>Non-stressed inoculated</td>
<td>20.60b (±0.52)</td>
<td>54.00b (±2.26)</td>
<td>51.33b (±4.50)</td>
<td>0.356b (±0.05)</td>
<td>60.20b (±1.06)</td>
<td>12.3ba (±0.04)</td>
</tr>
<tr>
<td>Drought-stressed uninoculated</td>
<td>24.80c (±0.32)</td>
<td>15.67ca (±0.30)</td>
<td>28.40ca (±0.69)</td>
<td>0.140ca (±0.02)</td>
<td>40.60c (±0.85)</td>
<td>8.16c (±0.65)</td>
</tr>
<tr>
<td>Drought-stressed inoculated</td>
<td>32.36d (±0.76)</td>
<td>63.30db (±9.95)</td>
<td>70.80d (±0.80)</td>
<td>0.389db (±0.09)</td>
<td>51.30da (±1.17)</td>
<td>9.20dc (±0.25)</td>
</tr>
</tbody>
</table>

*Sandhya et al. 2009, Biol. Fert. Soil*
Fig. 2 Neighbour-joining tree showing the phylogenetic relationship between *Pseudomonas* spp. (with GenBank accession numbers). The bars represent 0.002 substitutions per site, bootstrap values \( (n = 500) \)
Field trails with sunflower

under progress
Way forward

• Drought stress studies in maize
• Transcriptomic studies - Illumina HiSeq 2500
• Quantification of stress responsive genes – Real Time-PCR
Screening of the natural population from different dryland ecosystems used in the current study provided valuable strains, with multiple stress tolerance with a potential for use as efficient plant growth promoting rhizobacteria.

Drought stress tolerance in these microorganisms provide a biological understanding of the adaptation and survival of microorganisms in extreme environments.

Osmolyte production by these drought tolerant strains protected them against fluctuations in osmotic conditions which accumulated to higher levels to alleviate stress effects.

The accumulated osmolytes enhance the stability of proteins, cellular enzymes and membranes by replacing water around macromolecules under water limiting environments indicating osmotic cellular adaptation and helping the cell producing PGP phytohormones under stress conditions.

Inoculation showed a significant improvement in growth of crops tested under non-stress and drought stress.

The ability of these strains to produce IAA, gibberellins and cytokinins under osmotic stress could account for stimulating the plant growth and help in alleviating drought stress.

In addition to general plant growth, IAA and Cytokinins stimulates stress tolerance in plants.

Siderophore production by the drought tolerant isolates contributes iron nutrition of plants under drought stress.

Conclusion
Presence of ACC deaminase in the isolates may alleviate the drought stress effect by decreasing the levels of ethylene and promote longer roots, helping plants to survive under drought stress conditions.

Copious amount of exopolysaccharide production by the inoculated *Pseudomonas* spp. under stress conditions led to an organo-mineral sheath around these cells forming biofilm and increased aggregate stability and RAS/RT ratio and helped in the survival of plant under drought stress.

The electrolyte leakage is less in the inoculated seedlings under drought stress, indicating that due to inoculation increase in permeability of leaf membranes is less than control which is correlated with the accumulation of antioxidative enzymes.

Inoculation with drought tolerant *Pseudomonas* spp. compensate the drought effects and improve plant development through enhanced production of proline, amino acids, proteins and soluble sugars, which helps in maintaining cell water status, protects membranes and proteins from stress, thus resulted in better absorption of water and nutrients from soil.

Based on these studies we conclude that the isolation of indigenous microorganisms from the stress affected soils and screening on the basis of their ability to tolerate stress and effect of stress responsive mechanisms on PGP traits may be useful in the rapid selection of efficient PGPR strains that impart tolerance to crop plants against abiotic stress.
Acknowledgement

- Indian Council of Agricultural Research (ICAR), Govt. of India for providing the financial assistance in the form of network project on Application of Microorganisms in Agriculture and Allied Sectors (AMAAS)
- Science and Engineering Research Board (SERB), Department of Science and Technology, Govt. of India for providing the financial assistance in the form of network project “Differential expression of RNAs in maize under drought stress inoculated with ACC deaminase producing rhizobacteria”
- Central Research Institute for Dryland Agriculture, Hyderabad, Telangana State, India

Prof G.Pakki Reddy, Executive Director, Agri Biotech Foundation, Hyderabad, Telangana State, India
My Team

Thank you