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Phenomics approach to assess *soybean-Bradyrhizobium* symbiotic interactions under soil moisture stress
Beneficial Plant-Microbe Interactions

- Rhizosphere microbes
  - Dominant biological component
  - Ecological functions

- Evolutionary significance
  - Legume-rhizobium symbiosis
  - Mycorrhizal association
  - Plant adaptation to harsh environments
  - Integral plant Partners with mechanisms to induce tolerance

(Yang et al., 2009; Dimpka et al., 2010)
Soybean-drought tolerance

- Relevant drought tolerant traits
- Three strategies - improves plant physiological functions to water supply
  - Improved conservation of soil water
  - Increased crop access to water
  - Limited special sensitivities that affect yield under water deficit conditions e.g., Nitrogen fixation tolerance to drought (NFDT)

(Sadok and Sinclair, 2011)

- NFDT can be improved by
  - Identification of responsive soybean genotypes
  - Improving rhizobial nodulation competitiveness and N-fix efficiency
Rhizobial strategies on better legume symbiosis

- Fast growing rhizobia
  - *Rhizobium leguminosarum*,
  - *Mesorhizobium* sp. and others.
  - ACC deaminase activity

- Slow growing rhizobia
  - *Bradyrhizobium* sp.
  - Rhizobitoxine production

- Both mechanisms modulates plant stress ethylene synthesis during rhizobial infection and nodulation
Experimental details

- NRC-37, a susceptible cv. to drought stress
  - 8 treatments X 6 replications
  - Normal water & Low water conditions

- *Bradyrhizobium* strains
  - *B. elekani* USDA 61
  - *B. elekani* USDA 61
  - *B. Japonicum* USDA 110
  - *B. liaoningense* Bl-D
  - *B. Japonicum* Bj-I
  - *B. Japonicum* Bj-K
  - *B. Japonicum* Bj-M

- Observations & data recording
  - IR and VIS imaging
  - SPAD, Fluorescence and chlorophyll content
  - Nodulation and Yield related parameters

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PCR confirmation of rtx A gene in *Bradyrhizobium* strains & Imaging of soybean plants
Method of IR and VIS Imaging
IR and VIS images of *soybean-Bradyrhizobium* symbiotic interactions

Visible images

- *Bradyrhizobium* strains (no *rtxA*)
- *B. elkani* type strain (with *rtxA*)
- *B. japonicum* type strain (with *rtxA*)

IR images
Standardization of New Image analysis software

1. Red pixels
   - Equation: $y = 0.0055x - 2.8041$
   - $R^2 = 0.8948$

2. Green pixels
   - Equation: $y = 0.005x - 2.5353$
   - $R^2 = 0.832$

3. Blue pixels
   - Equation: $y = 0.006x - 2.5112$
   - $R^2 = 0.8539$

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From the IR images

- Avg. No. of Blue pixels were significant in soybean nodulated with *B. elekani* USDA 61 & 94 and *B. Japonicum* USDA 110.

- *rtxA* strains keeps the plant canopy cool during low moisture condition than other non-*rtxA* strains

From the VIS images

- Avg. No. of Green pixels were significant in soybean nodulated with *B. elekani* USDA 61 & 94 and *B. Japonicum* USDA 110.

- This indicates the contribution of these strains to effective N-metabolism leading to higher chlorophyll and Greener canopy
Improved plant physiological traits

**Relative Chlorophyll by SPAD chlorophyll meter**

**Quantum efficiency of PS II by fluorescence meter**

- **Bradyrhizobial strains**: Cont, Bl-D, Bj-I, Bj-K, Bj-M, Be-61, Be-94, Bj-110
- **Watered vs Water stressed**: Comparison of Relative Chlorophyll (SPAD units) and Quantum efficiency (Fv/Fm) for different Bradyrhizobial strains under watered and water-stressed conditions.
## Improved plant physiological traits

### Wet lab estimation of Chlorophyll content (mg/g FW of leaves) by DMSO method

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Watered Chl ‘a’</th>
<th>Watered Chl ‘b’</th>
<th>Watered Chl ‘a+b’</th>
<th>Watered Chl ‘a’</th>
<th>Watered Chl ‘b’</th>
<th>Watered Chl ‘a+b’</th>
<th>Water stressed Chl ‘a’</th>
<th>Water stressed Chl ‘b’</th>
<th>Water stressed Chl ‘a+b’</th>
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<td>Control</td>
<td>1.040&lt;sup&gt;c&lt;/sup&gt;</td>
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</tbody>
</table>
Nodulation and yield parameters

Nodulation competitiveness and efficiency

Pod numbers per plant
Improved yield parameters

- **Pods weight (g)/plant**
  - Cont, BL-D, Bj-I, Bj-K, Bj-M, Be-61, Be-94, Bj-110
  - Watered vs. Water stressed

- **Total seed weight (g)/plant**
  - Cont, BL-D, Bj-I, Bj-K, Bj-M, Be-61, Be-94, Bj-110
  - Watered vs. Water stressed

- **100 Seed weight (g)**
  - Cont, BL-D, Bj-I, Bj-K, Bj-M, Be-61, Be-94, Bj-110
  - Watered vs. Water stressed

Bradyrhizobial strains:
- Cont
- BL-D
- Bj-I
- Bj-K
- Bj-M
- Be-61
- Be-94
- Bj-110

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High nodulation efficiency of rtx producing *Bradyrhizobium* strains improved physiological and yield performance of soybean under limited soil moisture conditions

Simple phenotyping techniques like RGB based analysis of IR and VIS images of aerial parts could differentiate symbiotic interactions of *Bradyrhizobium* strains in soybean
Further works....

- Use of phenomic tools to assess beneficial plant-microbe interactions (symbiotic or free-living) and to identify potential candidates in large screens.
- Developing root phenotyping techniques to study beneficial plant-microbe interactions.
- Improvements and standardization of techniques – both imaging and analysis is in progress to enhance signal / noise ratio for higher precision.
Low cost image capture system
Acknowledgements

- Organizers of 3rd IPPS
- Members of IPPN
- Colleagues at NIASM
- ICAR

Thank you all