Genetic diversity and symbiotic efficiency of Rhizobia nodulating diverse lentil (*Lens culinaris* L.) germplasm

Third International Congress Biotechnology at the Service of Morocco Biotech 2020 Fez, February 27 - 29, 2020

Presented by: Sijilmassi Badreddine

Supervised by: Pr. Abdelkarim FILALI-MALTOUF (FSR)
Dr. Ahmed Amri (ICARDA)
Microbiology platform has been established to ensure:

- the multiplication, the conservation and the distribution of the collection (1380 ICARDA-Rhizobium strains).
- Research related to Rhizobial symbiotic efficiency of Food legumes in the dry areas.
Plan of the presentation

Genetic diversity and symbiotic efficiency of Rhizobia nodulating diverse lentil (*Lens culinaris* L.) germplasm

- State of the art.
- Objectives.
- Methodology.
- Findings and discussion.
- Conclusion and perspective.
• Symbiotic Nitrogen Fixation is the best natural solution for a sustainable nitrogen fertilization.
• However, Finding the best Rhizobia-legumes associations is still challenging.
• So far, research were mainly focused on screening the ubiquitous Rhizobia from different regions and soils.
• The introduced (exogenous) rhizobia failed mainly to nodulate once they are in competition with endogenous Rhizobia.
• Plant genotype is not taken into account?
• Choosing the best genotype for efficient Rhizobia-legumes association.
In order to bring more understanding of the Rhizobia-legumes association, the main objective of this study was:

to assess the genetic diversity and symbiotic compatibility of the rhizobial population nodulating lentil, collected at the same site from different genotypes planted under the same environmental conditions.

3 Factors:

- **Rhizobia** (to assess)
- **Location** (constant)
- **Plant genotype** (variable)
Methodology

10 Lentil germplasm

Isolation of 68 rhizobia

PCR-RFLP analysis of 16SrRNA and nodD genes

14 representative rhizobia

GENETIC DIVERSITY

SYMBIOTIC EFFICIENCY

Marchouch station

3 Varieties:
- Bakria
- Chakkouf
- Zaria

Growth parameters Assessment
- Number of nodules
- Total Nitrogen content
- Plant height
- Plant dry weight

Phylogenetic analysis
- 16S rRNA gene and rpoB, recA, gyrB house-keeping genes.
- nodD and nodA symbiotic genes.

Multi-locus-sequence-Analysis (MLSA)
- (16SrRNA-rpoB-recA-gyrB)
Findings and Discussion

Symbiotic Efficiency - Parameters of Growth

Variability of the Symbiotic efficiency

Number of nodules (A), Plant dry weight (B), Plant height (C) Total nitrogen content in leaves (D), in the three varieties of lentil (Bakria, Chakkouf, Zaria) in association with the selected isolates
Symbiotic Efficiency

Statistical Analysis

Based on the Parameters of growth with (P-value ≤ 0.05):

• 10/14 selected isolates had significant **symbiotic efficiency** on **Bakria variety**.
• 3/14 selected isolates had significant **symbiotic efficiency** on **Chakkouf variety**.
• 3/14 selected isolates had significant **symbiotic efficiency** on **Zaria variety**.

*Bakria* : Best adaptability with the Rhizobial population
Findings and Discussion

Phylogenetic trees built based on the individual analysis of the 16S rRNA (B), rpoB (E), recA (D) and gyrB (C) genes sequences of the selected isolates and the closest species. The trees were generated using MEGAX with Kimura 2-parameter (K2) distance model, with 1000 bootstrap analysis and bootstrap value (B.V ≥ 50%). The isolates were out grouped by Bradyrhizobium japonicum USDA 110.

● represents the sequences of the selected isolates.
Phylogenetic trees built based on the individual analysis of the *nodA* (A) and *nodD* (B) genes sequences of the selected isolates and the closest species. The trees were generated using MEGAX with Kimura 2-parameter (K2) distance model, with 1000 bootstrap analysis and bootstrap value (B.V ≥ 50%). The isolates were out grouped by *Bradyrhizobium japonicum* USDA 110. ● represents the sequences of the selected isolates.
Genetic Diversity - MLSA

Phylogenetic tree built based on the concatenated genes sequences *(16S-rpoB-recA-gyrB)* of the selected isolates and the reference *Rhizobium* species.

The strain 1159N52 is a potential new genospecies applying the threshold of 96% of the closest species (*R. leguminosarum*)

95% similarity
Findings and Discussion

Symbiotic efficiency

**Bakria variety** showed the best association with the isolated *rhizobia* strains in terms of plant growth parameters, followed by **Chakkouf** and **Zaria** varieties.

- Could be due to the adaptability and the coevolution of the genotype-rhizobia associations (Heath and Tiffin, 2007). **Bakria** is cultivated since 1982 in Morocco.
- Thus, The success of Symbiotic efficiency is mainly relied on the used **lentil variety**.

Genetic diversity

- The Phylogenetic analysis showed that the selected isolates were mainly closed to *Rhizobium laguerreae* and *Rhizobium leguminosarum*. But the difference was mainly at the **strain** level.
- One isolate **938N3** showed 100% similarity with *Mesorhizobium Huakii* ATCC 33669T with **nodD gene** sequences similar to those of *symbiovar viciae* strains within the species *Rhizobium leguminosarum* and *Rhizobium laguerreae*: Horizontal Genetic Transfer (HGT) could have been occurred between *Rhizobium* and *Mesorhizobium* genus.
Conclusion and Perspective

• Almost all the lentil germplasm were nodulated by strains belonging mainly to *R. leguminosarum* and *Rhizobium laguerreae* species. However, preference at the strain level was noticed between the ten accessions of lentil.

• Surprisingly, some lentil germplasm had **larger symbiont spectra** allowing them to be in association with rhizobia from different genera, such as *Mesorhizobium* genus.

• The results of the symbiotic efficiency test of the three Moroccan varieties showed that the selection of the **symbiont-legume association** should start by selecting the best genotype in terms of agronomic traits including its ability to nodulate with large spectra of *Rhizobia*.

• To bring more understanding, **rhizobia-legume association** should be assessed with the same **lentil genotypes** in **different geographical** places and under different **environmental conditions**.
• This research is funded through the GIZ-attributed funding to the Genetic Resources Section at ICARDA.

• We would like to thank:
  • Dr. Caludio Zucca-ICARDA Scientist, for providing data on soil analysis,
  • Mr. Hafid Aberkane-Senior Research assistant at ICARDA.
  • Mr. Kamal Hejjaoui-Senior Research assistant-Lentil Breeding, Lentils at ICARDA.
  • Mr. Adil El-Baouchi-Senior Research Assistant - Cereal and Legume Quality Testing at ICARDA.
  • Hassan Boulahyaoui-Ph.D. Student in Center of Genomics of Human Pathologies (GENOPATH), Faculty of medicine & pharmacy; Mohamed V University, Rabat, Morocco
  • Mr. Ayman Kricha-Ph.D. Student in Biochemistry and Molecular Immunology at the Huazhong University in CHINA.
  • Miss. Kenza Boubekri- Ph.D. Student Mohammed VI Polytechnic University (UM6P), Benguerir-Morroco.
Presented by:
Sijilmassi Badreddine

Supervised by:
Pr. Abdelkarim FILALI-MALTOUF (FSR)
Dr. Ahmed Amri (ICARDA)