

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

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Rhizobium Laboratory of ICARDA-Morocco

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.**



Rhizobium Laboratory/Molecular Microbiology Platform



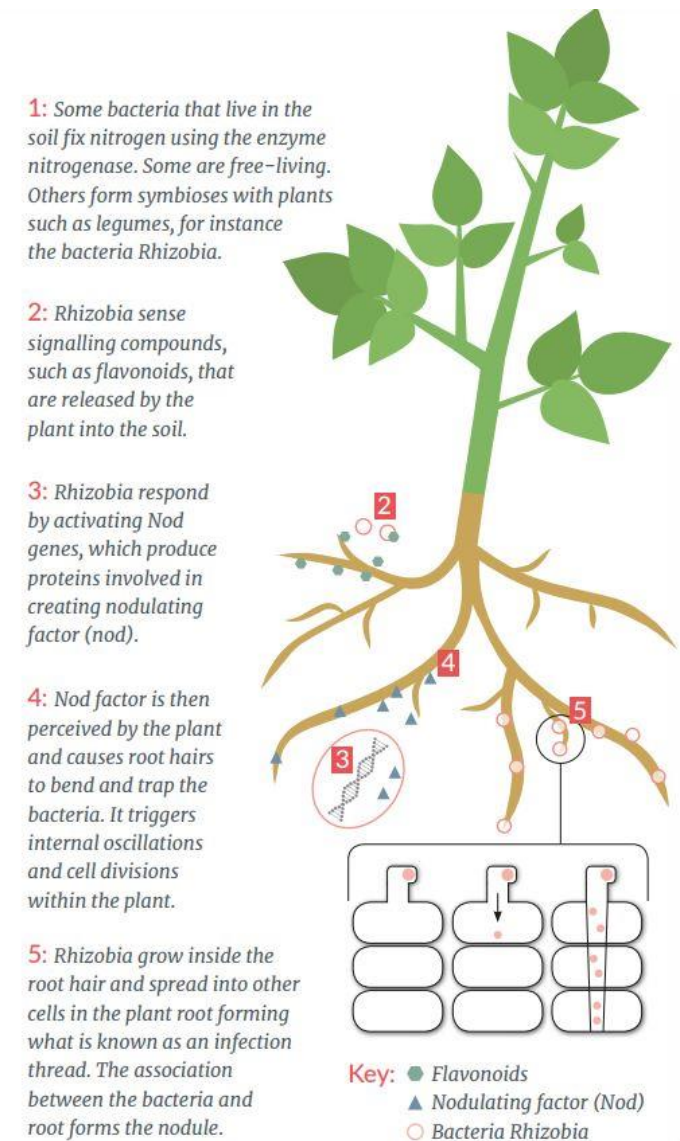
Plan of the presentation

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.

State of the art

- 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.**



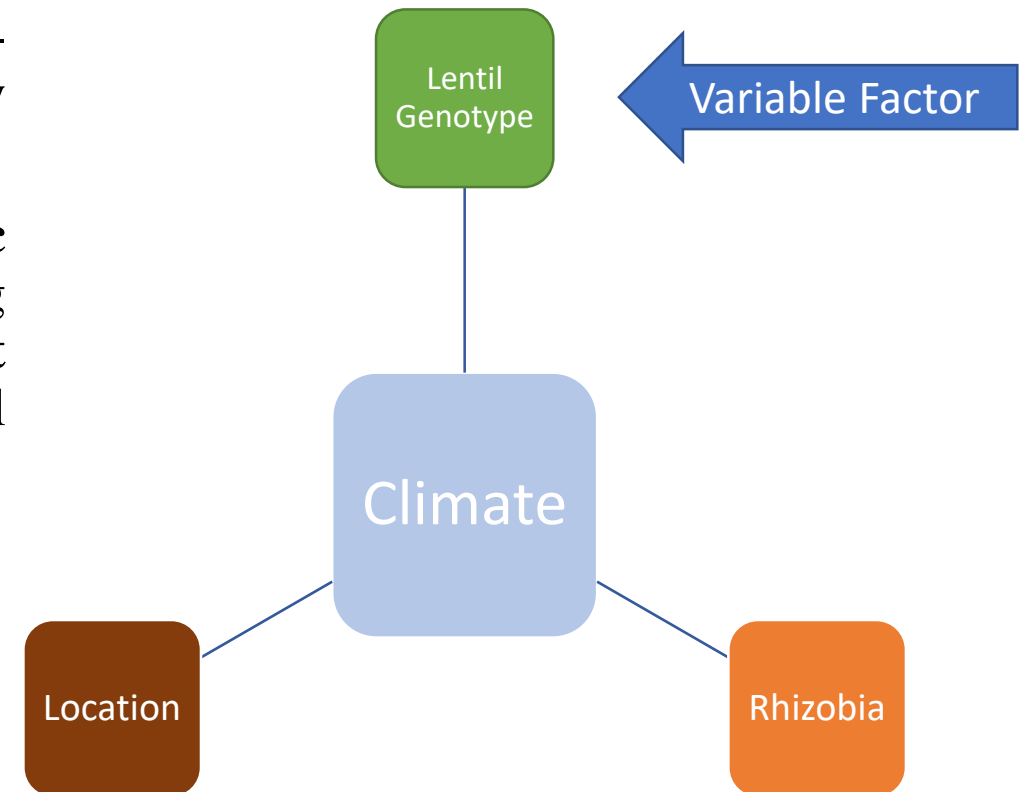
Objectives

In order to bring more understanding of the *Rhizobia*-legumes association, the main objective of this study was:

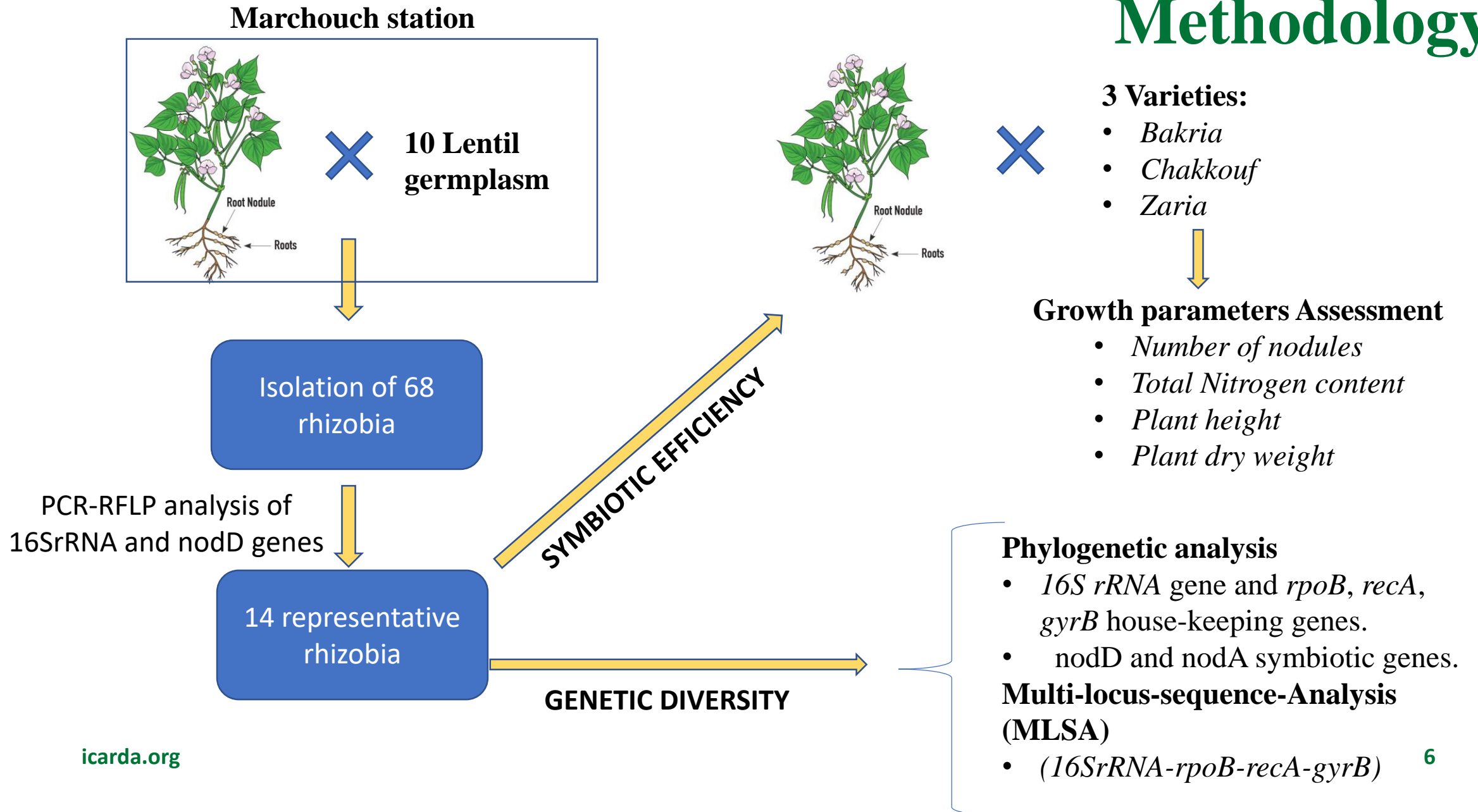
to assess **the genetic diversity** and **symbiotic compatibility** of the rhizobial population nodulating lentil, collected **at the same site** from **different genotypes** planted under the same environmental conditions.

3 Factors :

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

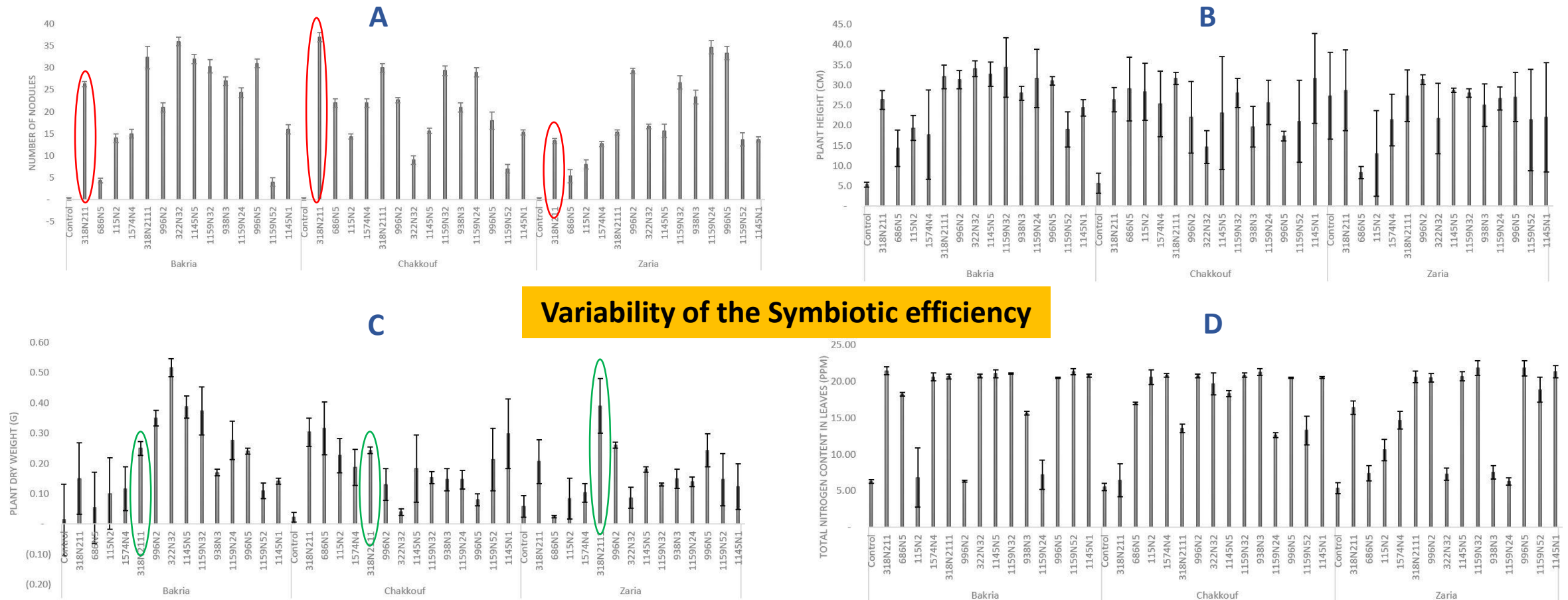


Methodology



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

Findings and Discussion

Symbiotic Efficiency

Statistical Analysis

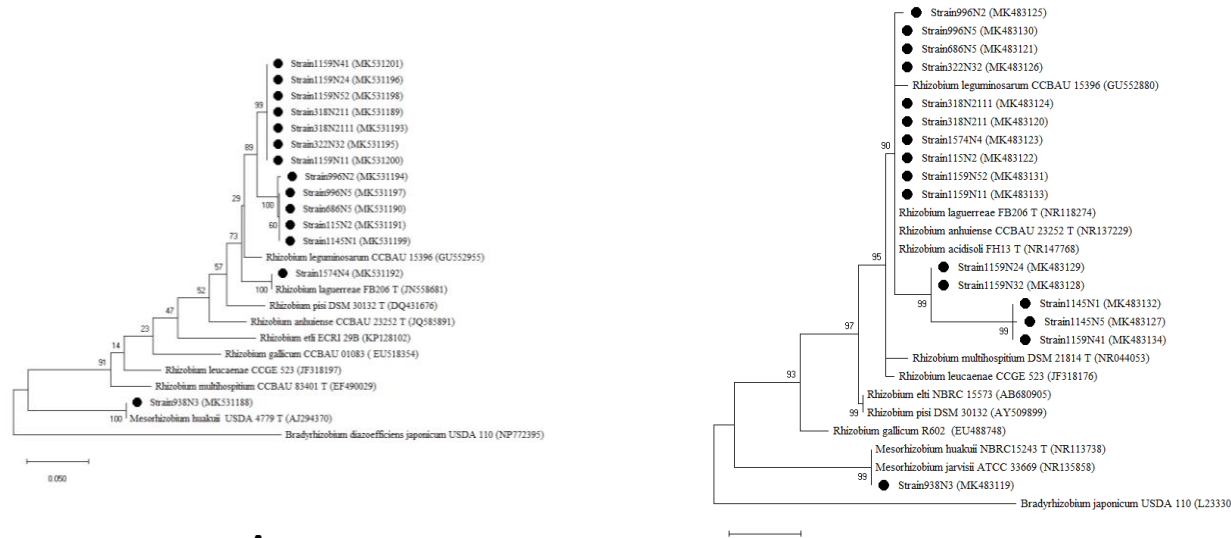
Based on the Parameters of growth with (P-value ≤ 0.05):

- **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

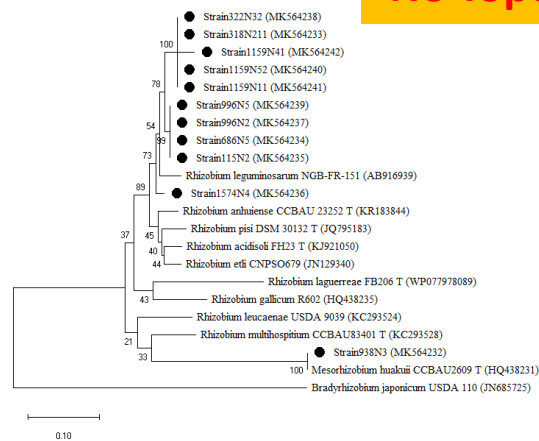
Genetic Diversity-16S rRNA and H-Keeping genes



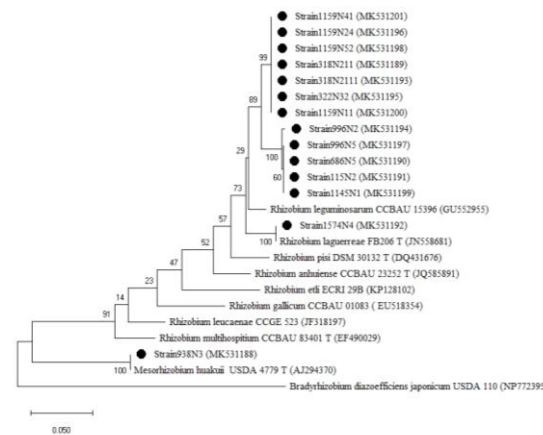
A

B

No Topological congruence



C



D

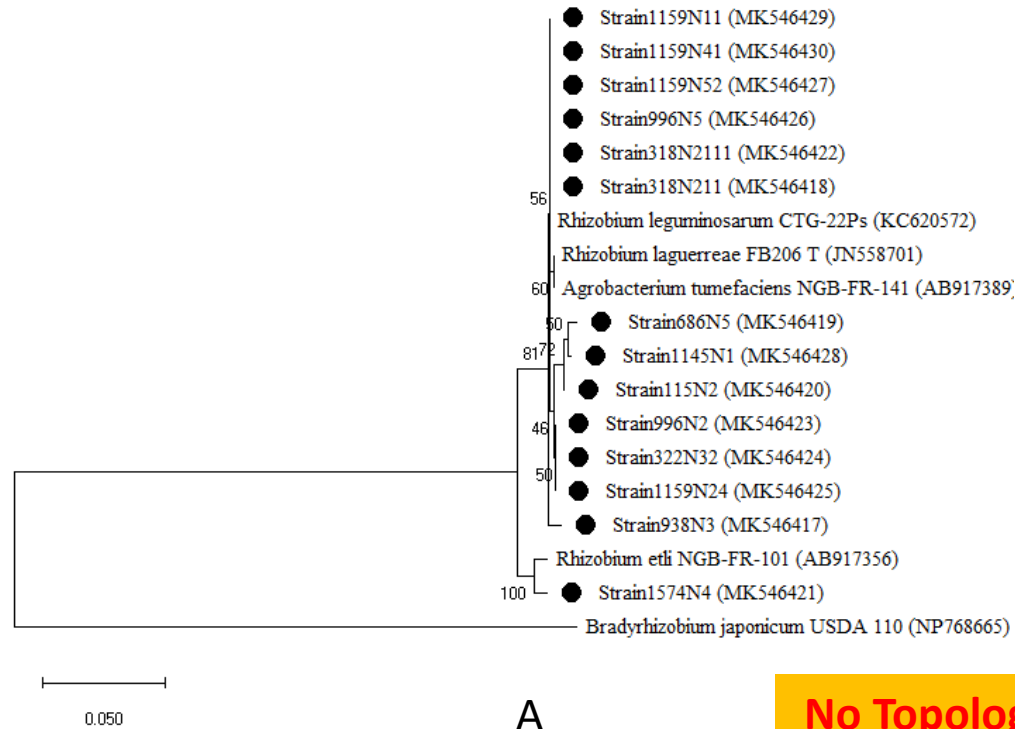
Phylogenetic trees built based on the individual analysis of the *16S rRNA* (B), *rpoB* (E), *recA* (D) and *gyrB* (C) genes sequences of the selected isolates and the closest species.

The trees were generated using **MEGAX** with Kimura 2-parameter (K2) distance model, with 1000 bootstrap analysis and bootstrap value ($B.V \geq 50\%$). The isolates were out grouped by *Bradyrhizobium japonicum* USDA 110.

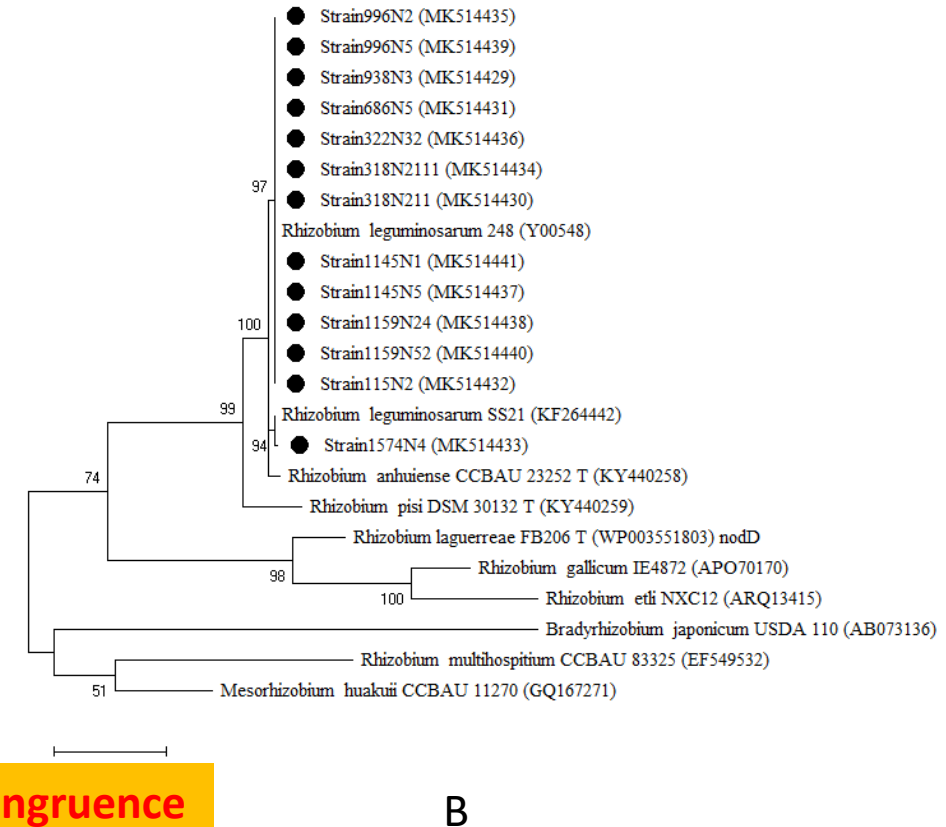
● represents the sequences of the selected isolates.

Findings and Discussion

Genetic Diversity- Symbiotic genes



No Topological congruence

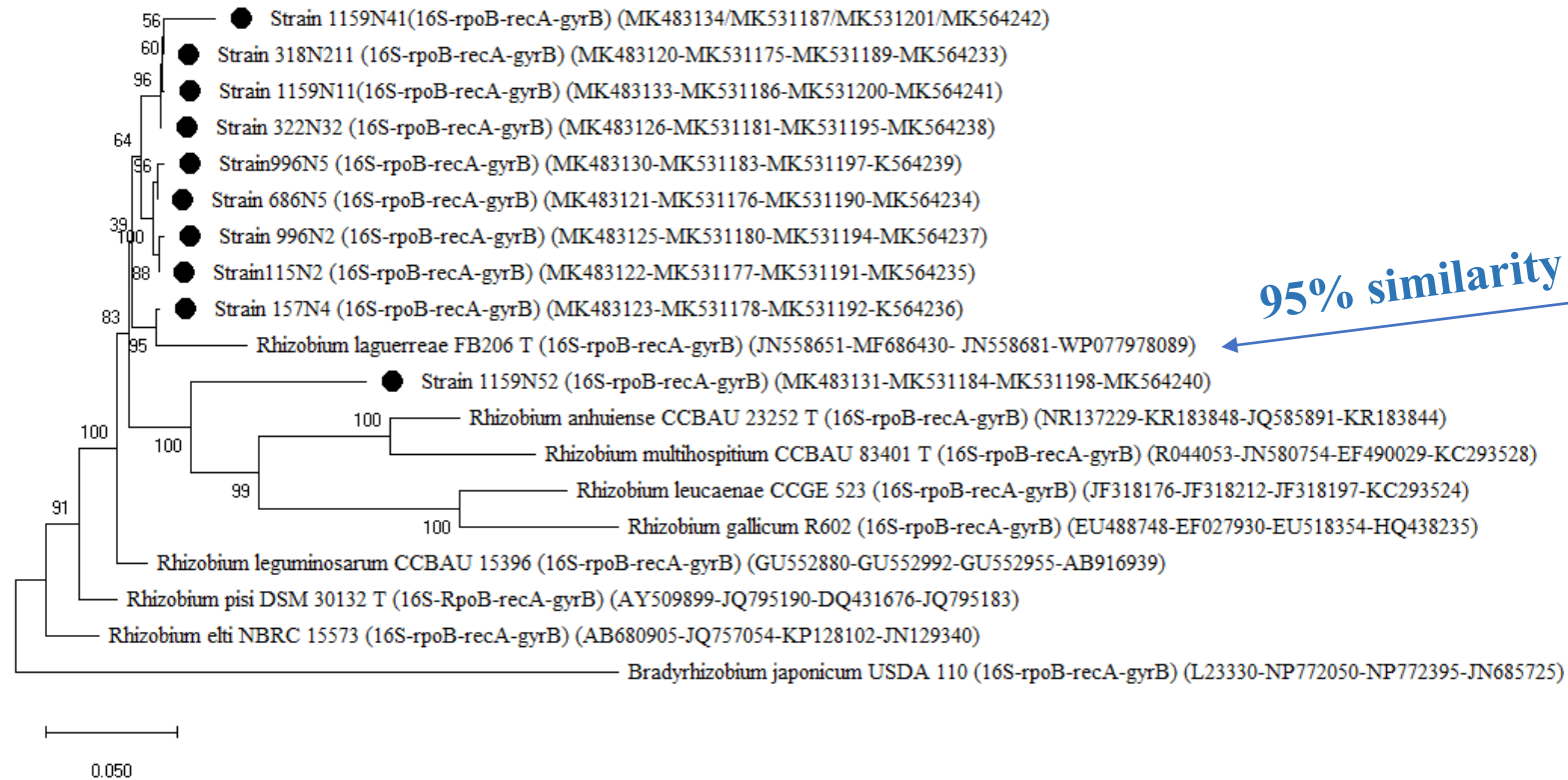


Phylogenetic trees built based on the individual analysis of the *nodA* (A) and *nodD* (B) genes sequences of the selected isolates and the closest species.

The trees were generated using **MEGAX** with Kimura 2-parameter (K2) distance model, with 1000 bootstrap analysis and bootstrap value (B.V $\geq 50\%$). The isolates were out grouped by *Bradyrhizobium japonicum* USDA 110. ● represents the sequences of the selected isolates.

Findings and Discussion

Genetic Diversity-MLSA



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

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

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 Huakuii* 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**.

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- **We would like to thank :**
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- **Miss. Kenza Boubekri**- Ph.D. Student Mohammed VI Polytechnic University (UM6P), Benguerir-Morocco.

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